DEVELOPMENT OF A BIO-SENSING TECHNIQUE FOR THE DETECTION OF PRIONS IN FOODS

A Thesis

by

ASHISH ANAND

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2003

Major Subject: Biological and Agricultural Engineering

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Approved as to style and content by:

Rosana Moreira (Chair of Committee)	Elena Castell-Perez (Member)
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ABSTRACT

Development of a Bio-Sensing Technique for the Detection of Prions in Foods.

(December 2003)

Ashish Anand, B.Tech. , Kanpur University

Chair of Advisory Committee: Dr. Rosana Moreira

An affinity based bio-sensing technique was developed using an anti-transmissible spongiform encephalopathy monoclonal antibody as a bio-recognition molecule. Fluorescein iso-thio-cynate (FITC), labeled with a prion epitope (QYQRES), was used as a decoy for prions. Experiments done in 0.1M phosphate buffer revealed that the dye fluorescence increased with the pH of the buffer and was influenced by solvent polarity.

Binding studies conducted at pH 6, 7, and 8 showed that the optimum pH for the antibody-decoy binding was 7. Maximum differences between control and antibody samples were observed at pH 7. The optimum incubation time was found to be less than 4 hours for the control, antibody, and the prion samples at room temperature. Prion detection curves were established at 4 and 10 nM antibody decoy concentrations. The lowest detectable prion concentration in phosphate buffer was 8 nM.

Experimental conditions determined in the phosphate buffer were used to implement the technique in gelatin and baby formula. Prion detection curves

were generated in 0.01, 0.4, 1.0 and 2.0 mg/ml of gelatin solution. The gelatin interfered with the binding and the displacement reaction of antibody, decoy and prion. Addition of an anionic surfactant, sodium dodecyl sulfate (SDS) at 0.3 mg/ml to gelatin samples facilitated prion detection in gelatin. The lowest detectable concentration of prion in gelatin was 0.5 nM at 0.4mg/ml gelatin. The baby formula samples produced light scattering and the intrinsic peak of baby formula at 526nm interfered with the dye peak at 514nm. Serial dilutions of baby formula were done to reduce the interference. Prion detection curves were then obtained at 1.31 and 5.34 mg/ml baby formula and 0.454 mg/ml of Triton-X-100 was added to the baby formula samples. The lowest detectable concentration of prion was 2 nM for baby formula.

This developed bio-sensing technique can be used to detect prion in gelatin and baby formula solutions. Addition of surfactants assisted prion detection in foods, while high concentrations of gelatin and baby formula had an adverse effect on the detection system.

DEDICATION

I dedicate my thesis to:

My parents, my wife, my brother and all my relatives

Without their love, support and constant encouragement, I would not have been able to complete this work.

ACKNOWLEDGEMENTS

First of all I would like to thank my advisor Dr. Rosana Moreira for her advice, support and belief in my ability to do this project. Thank you for allowing me to be a part of the food engineering program at Texas A&M and for helping me constantly in completing my degree. Your dedication to conduct research in food engineering is inspiring.

I would also like to thank Dr. Theresa Good for her invaluable advice in designing the experiments, explaining the concepts and always keeping my hopes alive. Thanks a lot Dr. Good; it would not have been possible without your help. I would also like to thank Dr. Gerard Cote for helping me with the optical engineering and critical analysis of the data. Your advice always brought significant improvements in the project. I would also thank Dr. Elena Castell for great suggestions and wonderful kindness.

To my lab colleagues James, Mustafa and Brad, I would like to thank you for your support and for always bringing a smile to my face in tough times.

My special thanks to my wife Sapna. It would not have been possible to complete this program without your support, care and morale boosting.

Last but not the least, I would like to express my appreciation to my parents, younger brother Anurag and my family in India for their constant support and good wishes.

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CHAPTER I

INTRODUCTION

Few diseases have captured both scientific and public attention this decade and among all of them, one of the most fatal neurodegenerative disorders is classified as Transmissible Spongiform Encepahlopathy. The transmissible spongiform encephalopathy (TSEs) (in both human and animals) has been studied with lot of emphasis on bovine spongiform encephalopathy (BSE) because of the spread of the disease in cattle, which also killed many people in the United Kingdom (‘mad cow disease’) (Fishbein, 1998).

BSE first appeared in the UK in 1985 (Brenig, 2001) and since then it has effected human life at health, scientific, economical, political, and judicial levels (Bounias, Purdey, 2002). In the UK BSE was known as a scrapie-like disease with the presence of characteristic lesions in the brains of affected cattle and the appearance of neurological disorders in cattle (Fishbein, 1998).

It is reported that from 1988-1997 170,000 cases of BSE had been found in over 34,000 farms in the UK. It has been speculated that more than a million cattle infected with BSE may have entered the food chain (Anderson et al., 1996).

This Thesis follows a format and style of *Journal of Food Engineering.*

Over 140 patients with variant Creutzfeldt-Jakob disease (CJD) have died since 1995, probably by consuming BSE infected processed meat. Although most of the cases were reported in Great Britain, France, and Italy future cases may appear in any country where BSE exists, (Brown et al., 2003). No case has been reported in USA till date.

The infectious agent behind TSEs/ BSEs is a misshapen protein called prion (PrP^{SC}), typically found in the brain and spinal cord tissue. The prion protein that causes the transmission of TSEs is formed by the post translational and conformational change in the normal prion protein (Hope and Manson, 1991; Prusiner, 1982; Prusiner, 1991).

This protein could accidentally be mixed with foods such as sausages, meat in ground beef, etc. Some detection techniques are available to test abnormal prions in the brain of slaughtered animals, but there are no tests available to detect prions in food products. Prions are highly stable, they resist extreme conditions involved in food processing as freezing, high temperature pasteurization, and drying.

Changing feed policies and monitoring animal diet can control epidemic in animals, but in case of humans BSE has an unknown incubation period before infection becomes evident. Hence, the only way to protect the US food supply chain from BSE is to develop a rapid and accurate detection technique for prions in foods. No information is available in the literature for the detection of prions in more complex matrices like foods. Unpredictable interactions of bio-molecules

present in foods with anti prion antibodies can pose serious challenges to the current detection techniques.

The Food and Drug Administration (FDA) identified the need to develop a detection technique for BSE in foods and this research is aimed at developing a biosensor for prions.

The objective of this research was to develop a biosensor to detect prion and prion peptides in different food materials. The detection technique utilizes the principle of competitive immuno assay in conjunction with fluorescence spectroscopy. This sensing technique requires less time and relatively moderate skills

Specific aims of this research were:

1. Development of a biosensor for the detection of BSE (prion and prion peptides) in buffer.
2. Implementation of biosensor in food systems like gelatin and baby formula for the detection of BSE.

CHAPTER II

LITERATURE REVIEW

2.1 Prion Biology and Structure[1]

Prions are proteinaceous, infectious particles that are composed largely if not entirely, of an abnormal prion isoform designated PrP^{SC}. The PrP gene encodes both PrP^{SC} and the cellular isoform PrP^{c} by a posttranslational process (Prusiner, 1991). The transformation of PrP^{C} into PrP^{SC} involves a profound conformational change (Riesner et al., 1996). The PrP molecule has two domains that play different roles in the conversion of PrP^{C} into PrP^{SC} **(**Figure1) (DeArmond and Bouzamondo, 2002). First, there is a stable or ordered core domain that contains PrPs two asparagine- linked oligosaccharides; two A-helices, designated helix-B and helix-C, that are stabilized by a disulfide bridge between Cys^{179} and Cys^{214}; a phos-phatidylinositol glycolipid (GPI) attached to the C-terminus at residue 231 which anchors PrP^{C} to the plasma membrane; and the protein X binding sites, which are believed to lower energy barrier for conversion of PrP^{C} to PrP^{SC} when PrP^{C} binds to the protein X (Kaneko et al., 1997; Telling et al., 1995). Secondly, there is a variable or disordered domain that contains the portion of PrP^{C} that interacts with PrP^{SC} and changes its conformation from primarily unstructured in the former to β-sheet in the latter (Figure 2) (Prusiner et al., 1998).

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[1] Parts of section 2.1, 2.2 and figure 1, 2 are reprinted with permission from *Toxicology*, *181-182*, DeArmond, S., Bouzamondo, E., Fundamentals of prion biology and diseases, 9-16, copyright (2002) with permission from Elsevier.

PrP^{SC} is distinguished from PrP^{C} by its high β-sheet content, (PrP^{SC} is about 30% α-helical and 43% β-sheet and PrP^{C} is 42% α-helical and 3% β-sheet) (Figure 2) (Pan et al.,1993), protease resistance and insolubility (Baldwin et al., 1994; Pan et al., 1993; Pruisner et al., 1983; Safar et al., 1993). Limited proteolysis purification techniques suggest that the apparent molecular weight of PrP^{SC} is 27-30KDa. Proteolysis of PrP^{SC} produces an N-terminal truncated protein designated as PrP 27-30 under conditions in which prion infectivity is retained (Riesner et al., 1996). PrP 27-30 forms rod-shaped polymers with the tinctorial properties of amyloid that are referred to as prion rods (Prusiner et al., 1983). Electron microscopy suggests that prion rods are 20-30 nm in diameter and 50-120 nm in length (Nguyen et al., 1995). Treatment of prion rods with the detergents and solvents increased α-helix content of PrP^{SC} and resulted in a substantial reduction in scrapie infectivity (Gasset et al., 1993; Safar et al., 1994). It has been postulated that aggregation of prion leads to its infectivity.

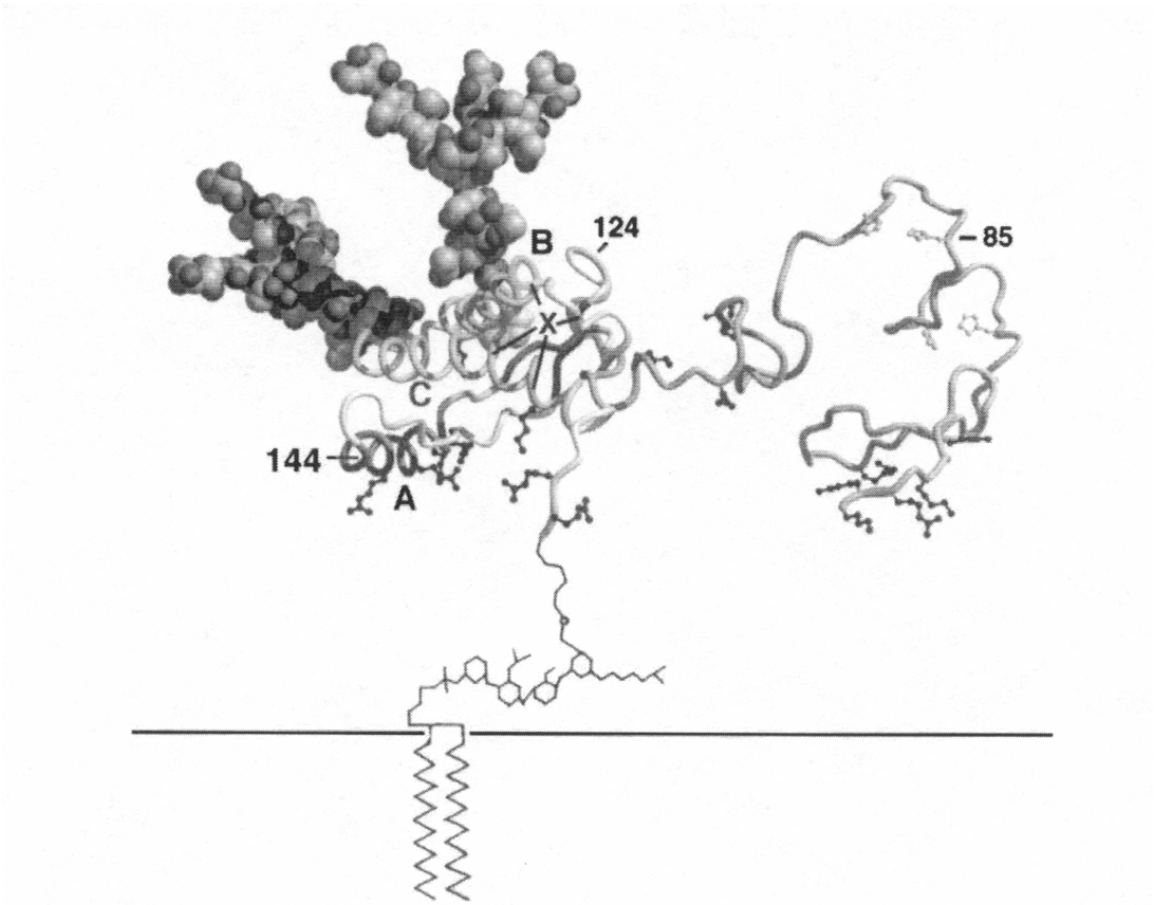

Figure 1: *Structure model of PrP^{c} molecule. PrP^{C} shown is attached to the plasma membrane by its GPI anchor to indicate how the range of movement of the N-terminal half of the molecule might be constrained in vivo. The putative protein X binding sites are indicated with an X with lines pointing to the discontinuous epitope on helices C and B with which it interacts (Kaneko et al., 1997; DeArmond et al., 2002).*

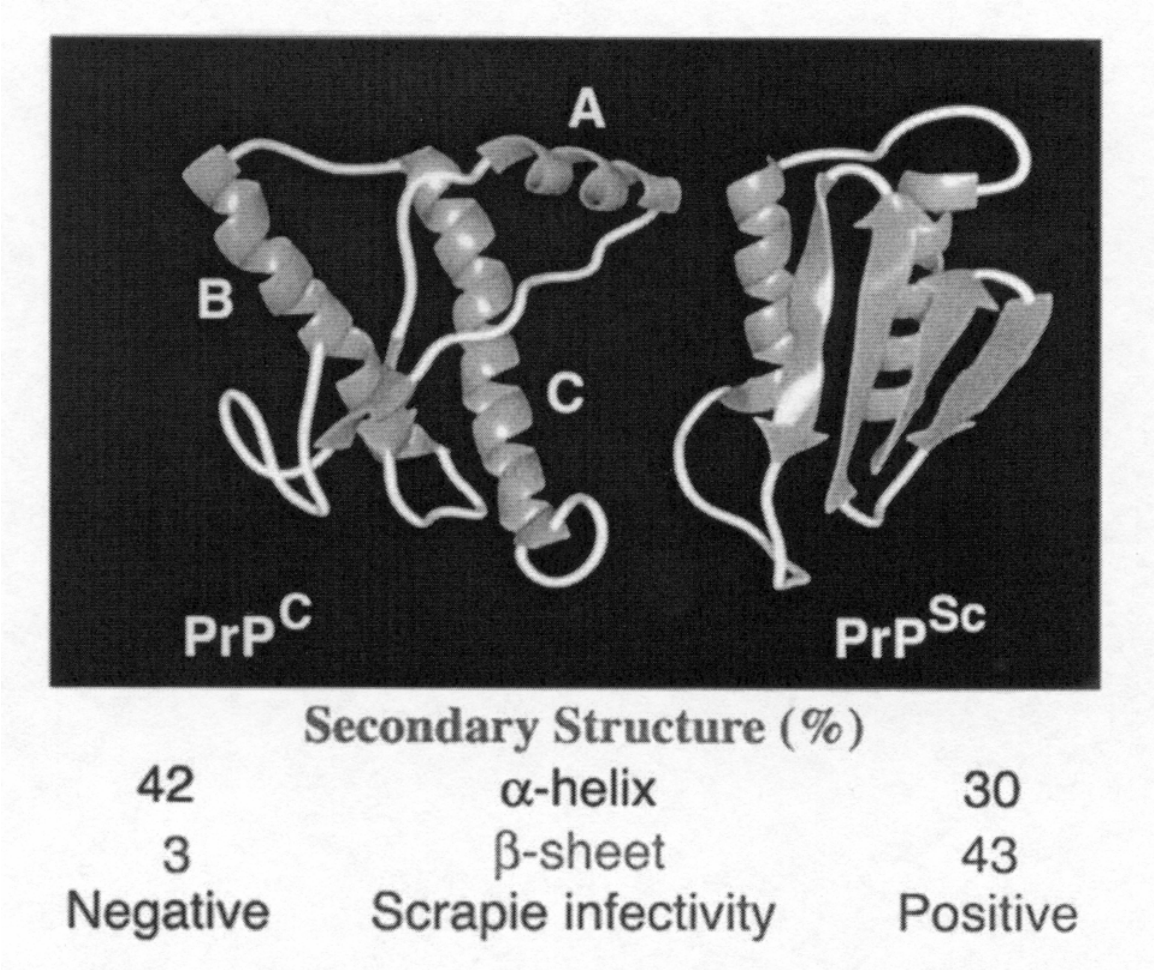

Figure 2: *Models of normal PrP^{C} and abnormal PrP^{SC} deduced from Syrian hamster recombinant PrP 90-231 (Prusiner, 2001).*

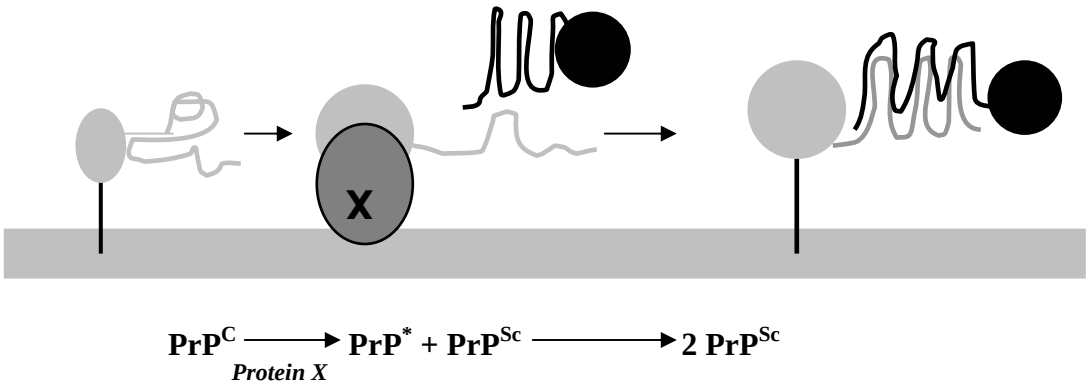

Figure 3: *Redrawn Schematic of the interaction of the N- terminal half of PrP^{C} with the N- terminal half of PrP^{SC} that results in the conversion of the non-infectious prion into infectious prion.*

2.2 Why Does Prion Disease Occur[1]

There are four major reasons for the existence of prion diseases (DeArmond and Bouzamondo, 2002). First, the mature, full-length prion protein molecule can exist in two conformations (PrP^{C} and PrP^{SC}) without an additional, demonstrable chemical modification (Prusiner et al., 1998). Secondly, regardless of its origin, PrP^{SC} can interact with PrP^{C} and cause the latter to adopt an identical β-sheet conformation which, in doing so, initiates a self perpetuating process that results in geometrically increasing PrP^{SC} concentrations and increasing prion infectivity titers in the brain (Figure 3) (Cohen et al., 1994; Jendroska et al., 1991).

Thirdly, some mutations of and some insertions in the prion gene cause small amounts of PrP^{C} to convert spontaneously to PrP^{SC}, which accounts for the familial CJD and FFI (Fatal Familial Insomnia) categories of human prion diseases (DeArmond and Bouzamondo, 2002). Fourthly, it was recently discovered that other mutations of the prion gene expressed in transgenic mice, specifically those that cause GSS (Gerstmann Syndrome) like syndromes cause small amount of PrP^{C} to adopt a pathogenic transmembrane topology (Hegde et al., 1998; Hegde et al., 1999). There is not much information available on the concentration of prion necessary for disease transmission. Hence in a safe food supply chain there should not be any traces of PrP^{SC}.

2.3 Current Detection Methods for BSE

Methods used for the detection of BSE are Western Blot, ELISA and 2-site sandwich assay (Grassi et al., 2000; Osech et al., 2000; Meyer et al., 1999) (Table1). Detection limits for these immunological methods are at 100pg/ml (approximately 40fM), which is sufficient to detect PrP^{SC} in the infected animals (Grassi et al., 2000).

No data is available to suggest that picomolar level of detection is good enough to prevent disease transmission after ingesting prion-contaminated food. An amplification procedure developed by (Saborio et al., 2001) on the basis of cyclic incubation and sonication of brain homogenates contaminated with PrP^{SC} mixed with homogenates of uninfected animals can increase the concentration of PrP^{SC} up to 100 fold. This procedure has not been tested in food products.

Detection of prions using above mentioned immunochemical techniques is more accurate than the histological examination of the brain. Many polyclonal and monoclonal anti-PrP antibodies have been developed with the aim of increasing sensitivity and specificity, but none of them are able to distinguish between PrP^{C} and PrP^{SC}. As a result there is a need of pre-treatment with proteases to remove or degrade PrP^{C} (Ingrosso et al., 2002). Among all the immunological methods, Western blotting is the best-characterized and widely validated technique. It has many disadvantages which include: lengthy

Table 1:

Current detection techniques for PrP^{SC} (Ingrosso et al., 2002)

Technique	Operation Principle	Detection Method	Detection Limit	References
Western Blot	SDS-PAGE, electroblot on a membrane, and the use of molecular sizes of prion.	Chemiluminescence	10-20pM	(Wadsworth et al., 2001); (Zanusso et al., 2002); Lee et al., 2000)
ELISA	PrP^{SC} absorption onto an ELISA well; incubation with anti-PrP specific primary and secondary antibodies.	Chemiluminescence	2pM	(Deslys et al., 2001); (Grassi , 2001)
DELFIA/CDI	PrP^{SC} absorption onto a plastic well; incubation with anti-PrP specific primary secondary antibodies.	Fluorescence	0.2-2pM	(Safar et al., 1998); (Volkel et al., 2001)
FCS	PrP is tagged by two fluorescent antibodies; highly fluorescent aggregates of PrP^{SC} are detected by confocal microscopy.	Fluorescence	2pM	(Giese, 2000; Bieschke, 2000)
MUFS	Conformation- specific emission scan of aromatic residue of Prp.	Fluorescence	In pM	(Rubenstein et al., 1998)

procedure and not many samples can be done in a single gel (Ingrosso et al., 2002; Kuczius and Groschup, 1999). Many of these limitations are eliminated by techniques like the sandwich enzyme linked immunoabsorbent assay (ELISA) (Deslys et al., 2001; Biffiger et al., 2002).

Dissociation-enhanced lanthanide fluorescence immunoassay/ conformation- dependent immunoassay (DELFIA/CDI) is an advanced immunoassay with an ELISA where the detection system is sensitive time-resolved lanthanide fluorescence instead of chemiluminescence (Safar et al., 1998; Macgregor and Drummond, 2001; Volkel et al., 2001). Detection limit using this method can be as low as picograms of PrP^{SC} per ml therefore it is one of the most sensitive techniques for the detection of prion. However, similar to other immunological detection techniques, DELFIA/CDI has a limitation to differentiate between non-infectious and infectious prion (Ingrosso et al., 2002).

At present, there are only two methods that can prevail without the availability of specific anti PrP^{SC} antibodies. One is the multi-spectral ultraviolet fluorescence spectroscopy (MUFS) (Rubenstein et al., 1998) and the other is the confocal dual-color fluorescence correlation spectroscopy (FCS) (Giese et al., 2000; Bieschke et al., 2000). MUFS utilizes the presence of aromatic amino acid residues in the protein and identifies protein on the basis of their characteristic emission spectrum. Though no recent work has been done using MUFS, it is capable of discriminating cellular prion from the infectious one and various strains of PrP^{SC} (Brown et al., 2001). FCS identifies single fluorescent molecule

as they pass through laser beam and the objective of confocal microscope with a photon counter. This technique needs very small sample volumes and it is 20 times more sensitive than the Western blot (Bieschke et al., 2000). However results obtained from FCS need validation, as the instrument is not available in other laboratories.

All the above-mentioned techniques are sensitive and selective for the detection of PrP^{SC}, but there are limitations associated with them, Western blot is time consuming and requires high expertise (Ingrosso et al., 2002); DELFIA/CDI cannot differentiate between normal and pathological prion, no further development has been done in case of MUFS after 1998, therefore practical applicability of MUFS is doubtful in near future (Ingrosso et al., 2002).

Hence it is imperative to develop an alternative rapid and relatively simple detection technique that can differentiate between infectious and non- infectious prions.

2.4 Food Safety and BSE

Outbreaks of BSE in cattle and resultant deaths of more than 140 individuals due to consumption of BSE infected beef have created great health concerns among consumers, scientists, and government officials. Hence it is imperative to protect the food supply chain with increased assurance and supervision (Fishbein, 1998). To protect the public health from the threat of BSE a number of guidelines has been issued by the World Health Organization (WHO), Organization International des Epizootes (OIE), the Food and

Agricultural Organization (FAO) as well as by national and regional governments and organizations (European Union) (Fishbein,1998).

Basically these measures fall into three categories: (a) To avoid BSE-contaminated animals and meat products entering the food supply chain or being used in the production of medicinal products; (b) to screen and supervise BSE and CJD; (c) to identify research areas and efficient implementation of the findings (Dora, 1998).

In the above context WHO-(1996) issued the following recommendations to protect the public health and ensure safety of the food supply chain:

1. No part or product of any animal that has shown signs of transmissible spongiform encephalopathy TSE, or tissues, which are likely to contain BSE agent should enter the food chain.
2. All countries should establish surveillance and compulsory notification of BSE.
3. All countries should ban the use of ruminant tissues in ruminant feed.
4. Gelatin and tallow are only considered safe if effective rendering procedures are used.
5. Medicinal products and medical devices should be obtained from countries with no sporadic cases of BSE and measures recommended to minimize the risk.

On the basis of these regulations gelatin can only be considered safe if adequate measures have been taken during gelatin manufacturing. In the US, the source of raw materials for gelatin manufacture is exclusively from beef hides, beef bones, and pork skin. Therefore, food safety risk for the foods made from gelatin can be high if gelatin is contaminated with BSE. To minimize the risk of contamination for the products made from gelatin it is important to detect the presence of prions in gelatin.

2.5 Gelatin

It is a common product with a wide field of application. Gelatin is used for quality improvements in foods and medicines. It also serves as a supplementary source of protein, stabilizer, and emulsifier. It is also used for flavor enhancement, clearing of drinks, and as a collagen source for dietetics (Fennema, O., 1996).

Gelatin is manufactured by a controlled hydrolysis from protein collagen. Gelatin is composed of a unique sequence of amino acids. Unique features of gelatin are the high percentage of the amino acids glycine, proline and hydroxyproline (Figure 4).

Production of gelatin can be summarized in the following steps:

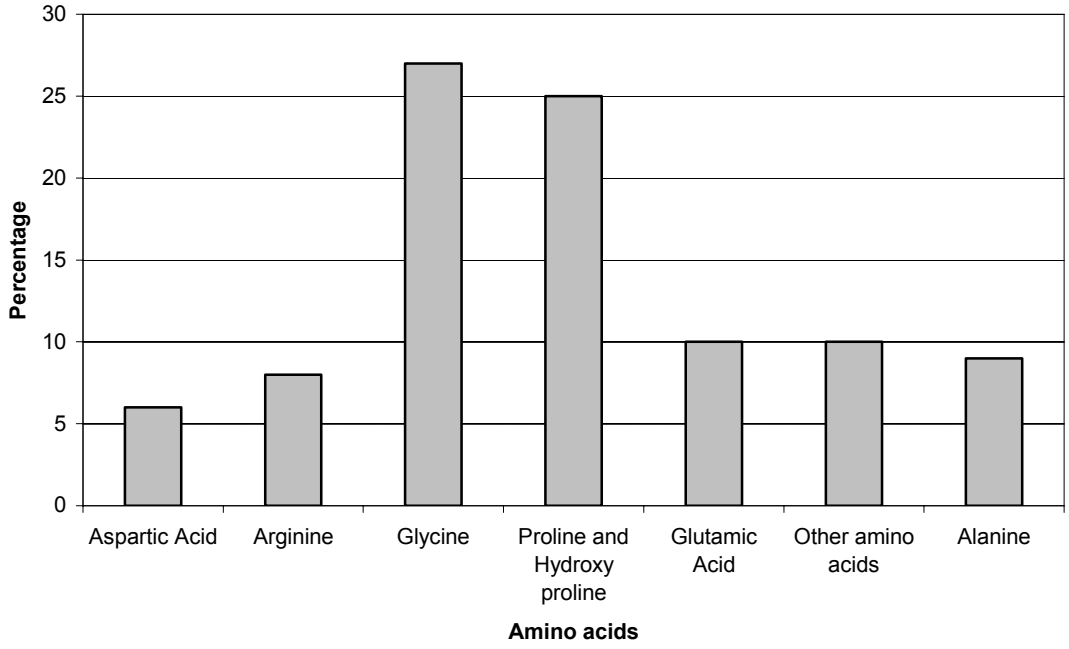

Figure 4: Redrawn amino acid composition of gelatin (www.gelatin.com).

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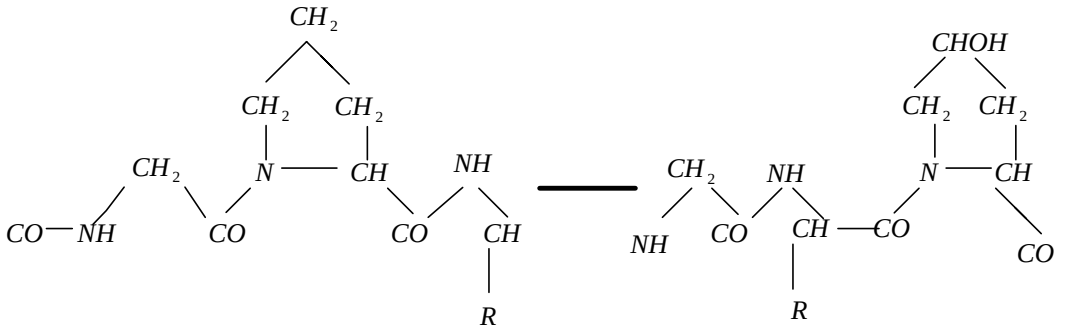

Glycine Proline Y Glycine X Hydroproline

Figure 5: Redrawn gelatin structural chain (www.gelatin.com).

Gelatin molecules contain repeating sequences of glycine-X-Y triplets, where X and Y are proline and hydroxyproline amino acids. These sequences are accountable for the triple helical structure of gelatin and its capability to form gels by immobilizing water (Figure 5).

1. Raw material for manufacturing is obtained from hidesplits and connective tissues and the bones of cattle.
2. Bones are splintered into pieces of less than 12mm diameter. They are kept in hot water to reduce the fat content to 2% and then dried for 30 minutes at 100°C (Schrieber and Seybold, 1993).
3. Phosphate minerals are removed from cleaned pieces by treating them with 4% hydrochloric acid, at a pH below 1.5 allowing collagen structure to be intact. This process takes 5 days, and begins with an already used, more diluted hydrochloric acid, which is replaced by a fresh batch of HCl at the end of the treatment (Schrieber and Seybold, 1993).
4. More than 90% of the gelatin won obtained from the cattle is treated additionally. It normally takes about 50days at pH greater than 12.5 to expose the cross connections between collagen molecules (Schrieber and Seybold, 1993).
5. Once gelatin is extracted step by step with water of increasing temperature, it is sterilized at 140°C for 4 seconds (Schrieber and Seybold, 1993).

The agents of scrapie and BSE are extremely resistant to physical and chemical treatments. Infectious agent can survive extreme acidic conditions, more intense than present in gelatin production; they are also resistant to alkaline conditions and drying temperature. In a nutshell the procedures used for gelatin production can reduce the infectiousness of BSE-contaminated raw material but complete inactivation is not possible. Therefore, we need a sensitive detection technique that can sense prion during the various processing steps of gelatin.

2.6 Collagen and Its Structure

Collagen is a protein, which is involved in the formation of connective tissue together with elastin and polysaccharides. It is also present in cartilage, tendons and ligaments. The shape of the collagen molecule is like a rigid rod, about 3000A° long, with a diameter of 14A° and a molecular weight of approximately 300,000 (Schwick and Heide, 1969).

Collagen contains small amounts of tyrosine and no tryptophan. The most common amino acids are glycine, proline, and hydroxyproline (Schwick and Heide, 1969). The general structure of collagen contain polar and apolar regions following each other. The most important amino acid sequence is glycine-proline-hydroxyproline and every third amino acid is generally glycine. The two different known peptide chains are; the $alpha_1$- and the $alpha_2$- chain, each consisting of about 1000 residual amino acids and differing little in their

sequence. The $alpha_1$ chain contains five and the $alpha_2$- chain seven subunits. The individual chains form a helix in which hydroxyproline and proline are turned outwards to avoid sterical imbalance (Secondary Structure) (Rich and Crick, 1961). The collagen molecule contains three helices; two $alpha_1$ and one $alpha_2$, which are twisted around a common axis in the form of a triple helix (tertiary structure). Helix is stabilized by the hydrogen bonds of inner glycine residues and the covalent bonding (Bornstein, 1966).

The quaternary structure of collagen (Fibrils) contains individual collagen molecule aggregate. Hydrogen bonds, van der Waal's forces, and covalent bonds play important role in stabilizing the structure.

Collagen is hydrolyzed into gelatin under certain denaturizing conditions, e.g. heating (Fessler and Bailey, 1966; Harding, 1965). The conversion into gelatin takes 30 minutes in a citrate buffer of pH 3.7 at 35°C. This leads viscosity reduction and breaking of collagen molecule into peptide chains (Figure 6 & 7) (Veis, 1964).

2.7 Baby Formula

Baby formula was first manufactured in Germany in 1867. The most common ingredients of formula were milk solids, wheat flour, malt flour and potassium bi carbonate. Nutrients requirements have changed a lot since 1867.According to FDA in (1985) the current formulation should have protein, linoleic acid, fat, choline, carbohydrate, inositol, calcium, phosphorous,

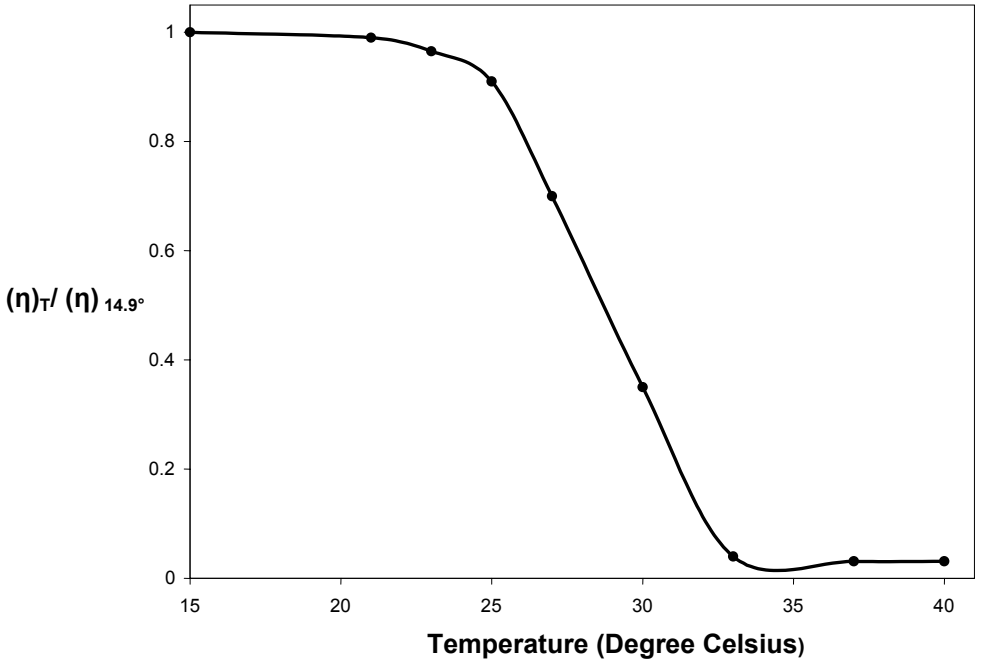

Figure (6): Redrawn collagen conversion to gelatin after heating for 30 minutes (Vies, 1964).

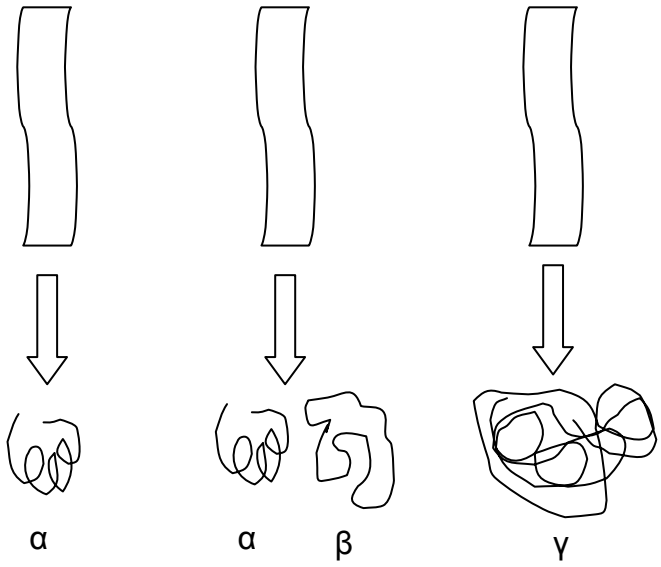

Figure (7): Redrawn breakdown of the collagen molecules into its subunits (Schwick, 1999).

magnesium, iron, zinc, manganese, copper, iodine, sodium, potassium, chloride, vitamin A, D, E, K, thiamine, niacin, pyridoxine, cobalamin, folic acid, biotin, panthothenic acid, ascorbic acid .

Most of the milk ingredients used in formulating a dry baby formula is used as supplements for protein, fat, carbohydrate etc. These ingredients are

made of milk produced by cattle; therefore it would be prudent to develop a detection technique for BSE (prions) in baby formula.

On the basis of the literature review it is evident that no data is available to test BSE in foods. Most of the available techniques are immunological and there compatibility with foods has not been tested. This research is focused on developing a detection technique that is rapid, user friendly, and can be implemented into food systems like gelatin and baby formula.

CHAPTER III

BIOSENSOR SCHEME

3.1 Macromolecule – Ligand Interactions

The role of every protein is related to its capacity to bind ligands (Brand et al., 1997). Binding of ligands to protein alters the three-dimensional structure and it influences the environment of an intrinsic or extrinsic fluor in the protein. These changes in structure can lead to quantifiable changes in the fluorescence spectrum (Sheehan, 2000). There are two possibilities s that can be used to investigate protein-ligand interactions. Either the fluorescence of the protein can alter on binding, or the fluorescence of the bound ligand can change. The changes in the fluorescence of a protein may be, due to one or more of the intrinsic tryptophan residues, because emission from this amino acid generally dictates the fluorescence of proteins. The other possibility is that the ligand fluorescence changes on binding. These changes can be accredited to energy transfer mechanism or by physical interaction of ligand with the protein and leading to change in the polarity of its surroundings.

Fluorescent probes to detect the presence of target analytes are commonly used in immunoassays (Perez-Luna et al., 2002). In general, an antibody, is required which interacts with the antigenic analyte followed by incubation with a labeled Detection of the target analyte can be done by bringing in labeled analogs of the analyte that compete for the same binding sites on the antibody (Competitive immuno-assay) (Perez-Luna et al., 2002). In general, an antibody, is required

which interacts with the antigenic analyte followed by incubation with a labeled reagent (Udenfriend, 1962; Kress – Rogers, 1997).

3.2 Antibody Their Structure and Function

Antibodies or immunoglobulin come under the category of glyco-proteins. They are produced by cells in response to an antigen. There are five major categories of antibodies, including IgG, IgD, IgA, IgE, and IgM (Steward, 1985).

The basic structure of immunoglobulins is shown in (Figure 8). Although antibodies can differ in structure, but are made from the same basic unit (Voet et al., 2001).

3.2.1 Heavy and Light Chain Antibodies have a Y shape structure as their basic unit. They are made of two identical light chains (Molecular weight 23 KD) and two identical heavy chains (Molecular weight 50-70 KD) (Steward, 1985).

3.2.2 Inter Chain Disulfide Bonds Heavy and light chains with the two heavy chains are held together by disulfide bonds and by non-covalent interactions. The number of inter-chain disulfide bonds can be different among different antibodies (Steward, 1985).

3.2.3 Intra Chain Disulfide Bonds They are present with in each of the polypeptide chains (Steward, 1985).

3.2.4 Variable and Constant Regions Heavy and light chains are divided into two regions based on the differences in their amino acid sequences.

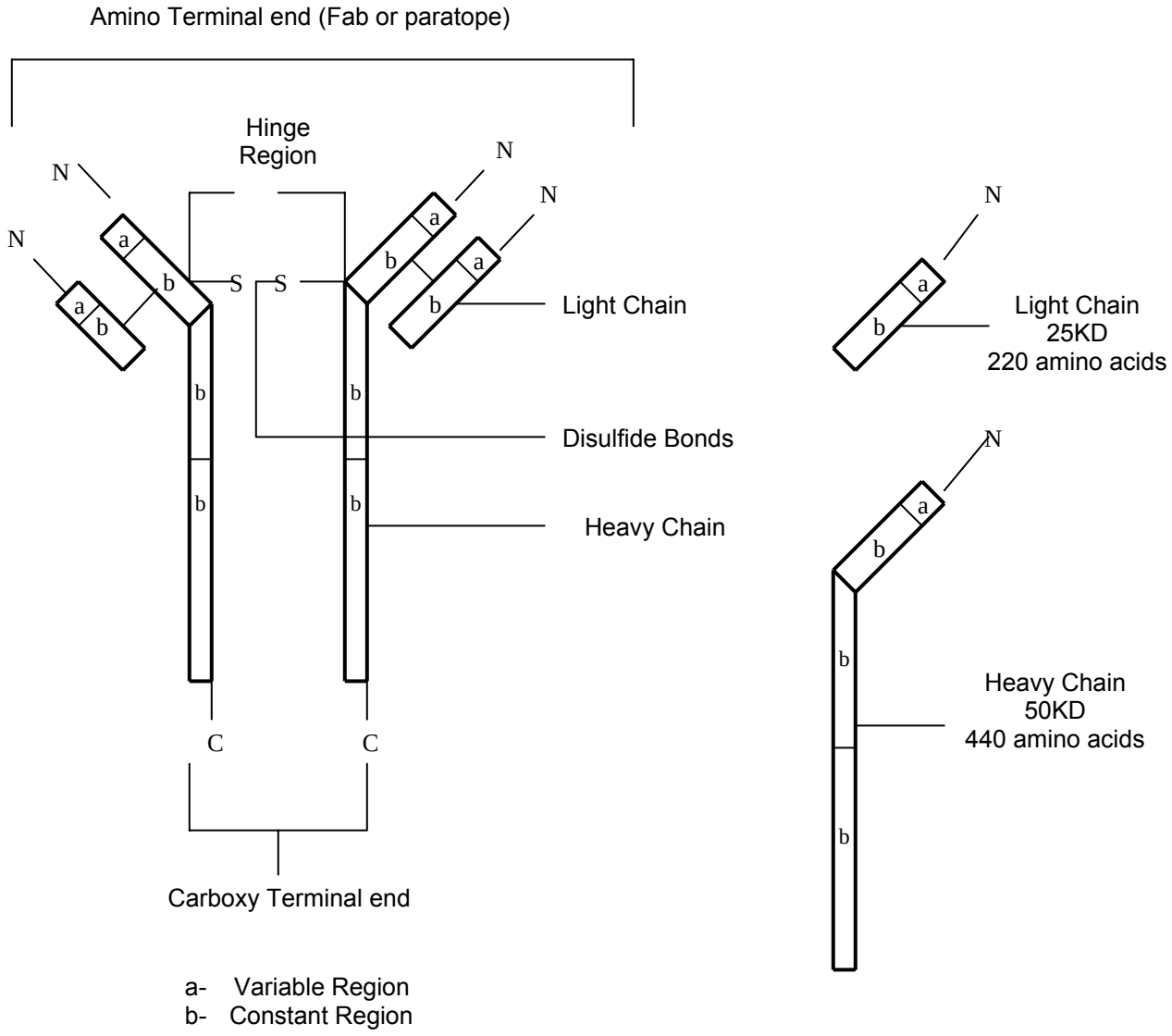

Figure 8: Redrawn general structure of antibody (Voet, et al., 2001).

Light chain contains 110 amino acids in the variable and constant regions but heavy chain is made of 110 amino acids in the variable region and 330-440 amino acids in the constant region (Steward, 1985).

3.2.5 Hinge Region It is the region where arms of the antibody molecule form Y and is called the hinge region since the molecule is flexible at this point (Steward, 1985).

3.2.6 Domains Three-dimensional structure of the antibody molecule reveals that it is not straight as shown in (Figure 8). Rather, it is folded into globular regions each of which has an intra-chain disulfide bond. These regions are called as domains (Steward, 1985).

3.2.7 Oligosaccharides In most of the antibodies carbohydrate molecules are present in the constant regions; in some cases carbohydrates may also be attached at other locations (Steward, 1985).

3.3 General Functions of Antibodies

Antibodies have two major functions, antigen binding and effector functions (Voet, Voet, 2001), (Steward, 1985).

3.3.1 Antigen Binding Antibodies bind specifically to closely related antigens. Each antibody has specific affinity towards a specific antigen. Binding to antigens is the primary function of antibodies. The number of antigens an antibody can bind refers to the valency of the antibody. Valency of the antibody can be as low as two and in some instances more than two (Steward, 1985).

3.3.2 Effector Functions Sometimes binding of an antibody to an antigen has no direct biological significance. The noteworthy biological effects are result of secondary “effector functions” of antibodies. The antibodies facilitate different effector functions by interacting with its antigen. The general effector functions are complement fixation and binding to different cells (Steward, 1985).

3.4 Antibody-antigen Interaction

The most important function of an antibody is form an antibody-antigen complex by interacting with its specific antigen. The interaction of antibody with the antigen results from the binding reaction of the binding site at antibody with the antigen.

3.4.1 Intermolecular Forces in Antibody-antigen interactions These forces are similar to the forces involved in the stabilization of protein configurations. They contribute to the stabilization of the antibody-antigen complex. The forces present in antibody antigen interactions generally are hydrogen bonding, hydrophobic interactions, ionic or columbic interactions, Van der Waal’s forces and steric repulsive forces (Steward, 1985).

3.4.1.1 Hydrogen Bonding Hydrogen bonding occurs when an H atom covalently linked to an electronegative atom with the unshared electron pair of another electronegative atom interacts. Amino or hydroxyl groups are the major hydrogen donors in antibody-antigen reaction (Steward, 1985).

-O… H----O—, -O… H----N--, -N…H----N—

3.4.1.2 Hydrophobic Interaction Non-polar side chains of amino acids do not form hydrogen bonds with water. These groups prefer to interact with each other rather than with water—this is called as apolar or hydrophobic interaction. These bonds are stable due to change in the structure of the aqueous environment, which excludes water when groups come together. The involved proteins go to lower energy state and gain entropy, resulting in an attractive force between them. These forces are very important in antibody-antigen reactions (Steward, 1985).

3.4.1.3 Ionic Interaction The interaction between oppositely charged groups on two side chains is termed as ionic interaction

$$R\text{----}COO^{-} \ldots\ldots {}^{+}NH_3\text{----}R$$

Where R is the protein side chain. These forces do not play major role in the stabilization of antibody-antigen complexes (Steward, 1985).

3.4.1.4 Van Der Waal's Forces When the electron cloud of two polar groups interacts. On account of this interaction induction of oscillating dipoles happens in the two molecules, which leads to an attractive force. The Van der Waal's forces vary inversely with the sixth power of the distance between the groups involved (Steward, 1985).

3.4.1.5 Steric Repulsive Forces Steric repulsive force comes from the interaction between non-bonded atom's electron clouds. Therefore, better complementarities of the electron cloud leads to lower repulsive force. This force is the leads to antibody discrimination. If the target analyte has an electron cloud

that is complimentary to that of the binding site, antibody will have low affinity for the antigen. Conversely, if the antigenic determinant and antibody are complementary then the antibody will have a high affinity because repulsive forces will be low (Steward, 1985).

3.5 Measurement of Antibody-Antigen Reactions

Generally, test for the measurement of antibody- antigen reactions depends on the interaction of the antibody with antigen, which leads to precipitation, which can be visualized in solution or gel. The reaction of an antibody (Ab) with an antigen (Ag) can be represented as follows:

$$Ab + Ag \underset{kd}{\overset{ka}{\Leftrightarrow}} Ab * Ag$$

Where k_a is the association constant, k_d is the dissociation constant. There are several methods are available to detect and quantify this interaction.

Table 2:

Classification of antibody detection methods (Steward, 1985).

Reaction	Analytical Method
Primary	Radio immnunoelectrophoresis
Primary	Farr binding
Primary	Antiglobulin technique
Primary	Fluorescence quenching, enhancement, polarization
Primary	Equilibrium dialysis
Secondary	Gel Diffusion
Secondary	P-80 radioprecipaitation
Secondary	Agglutination
Secondary	Complement Fixation
Tertiary	Passive cutaneous anaphylaxis
Tertiary	Arthus reaction
Tertiary	Immune elimination

3.5.1 Thermodynamics of the antibody-antigen reaction Binding affinity is the most important aspect of the thermodynamics of antibody-antigen reaction. Binding affinity refers to the strength of the interaction between an analyte and the antibody-binding site. Hence high affinity antibody refers to a strong complex formation with the antigen and visa versa. Affinity is a thermodynamic parameter and is expressed either in the form of equilibrium constant *K* or as the standard free energy ΔGº. The expression relating antibody and antigen at equilibrium is given by the following equation:

$$Ab + Ag \underset{k_d}{\overset{k_a}{\Leftrightarrow}} (Ab * Ag) \tag{1}$$

According to the Law of Mass Action, at equilibrium, the rate of forward reaction is equal to the rate of backward reaction

$$k_a(Ab)(Ag) = k_d(Ab * Ag) \tag{2}$$

$$\frac{k_a}{k_d} = K = \frac{[(Ab * Ag)]}{[(Ab)(Ag)]} \tag{3}$$

Where *K* is equilibrium constant, k_a is an association constant, k_d is dissociation constant.

Thermodynamic quantification of antibody antigen reactions requires that pure reactants in the solution. They should be homogenous with respect to antigens and binding sites. To meet these requirements monoclonal antibody is used, which in many cases behave ideally. Thermodynamic measurements with polyclonal antibodies are complexes because of their heterogeneous nature and multivalency for antigen.

Mass action model was used to determine the equilibrium constant in the present system of antibody-antigen reaction (Steward, 1985). Antibody—antigen reaction in the present biosensor falls under primary category and equilibrium conditions were measured by measuring the fluorescence intensity.

3.6 Fluorescence Spectroscopy[2]

Light, a form of electromagnetic radiation, propagates by waves. Light is characterized by a wavelength and frequency. When light impinges upon matter, two things can happen: it can pass through the matter with no absorption, taking place, or it can be completely or partially absorbed. In the later case energy is transferred to the molecule in the absorption process. The absorption of energy must occur in integral units called, quanta. Every molecule possesses a series of closely spaced energy levels and can go from a lower to a higher energy level by the absorption of a discrete quantum of energy. Only a few molecules are raised to a higher excited state and hence are capable of exhibiting luminescence (Munck, 1989).

3.6.1 Phenomenon of Fluorescence Luminescence is the emission of light from any substance and occurs from electronically excited states. Luminescence is divided into two categories, fluorescence and phosphorescence, depending on the nature of the excited state.

[2] Section 3.6 is reprinted with permission Lackowicz, J., (1999) *Principles of fluorescence spectroscopy*, Plenum Publishers, New York, USA.

In fluorescence the electrons that are in excited singlet state (the electron in the excited orbital is paired opposite to the second electron in the ground state orbital) return to the ground state by emitting photon.

The emission rates of fluorescence are typically 10^8 s^{-1}, so that the typical The emission rates of fluorescence are typically 10^8 s^{-1}, so that the typical fluorescence lifetime is near 10ns. Fluorescence typically occurs from aromatic molecules. Some typical fluorescent compounds are Quinine, Fluorescein, Rhodamine B, Acridine Orange, etc (Lakowicz, 1999).

Phosphorescence is emission of light from triplet-excited states, in which the electron in the excited orbital has the same spin orientation as the ground state electron. Transitions to ground state are forbidden and the emission rates are slow, therefore phosphorescence lifetimes are typically milliseconds to seconds (Lackowicz, 1999).

3.6.2 Fluorescence Life Times and Quantum Yields The fluorescence life times and quantum yield are perhaps the most important characteristics of a fluorophore. The quantum yield is the number of emitted photons relative to the number of absorbed photons. The higher the value of quantum yield, the greater the fluorescence of a compound. The lifetime is also important, as the lifetime determines the time available for the fluorophore to interact with or diffuse in its environment. The lifetime of the excited state is defined by the average time the molecule spends in the excited state prior to return to the ground state (Lakowicz, 1999).

3.6.3 Fluorescence Spectrum Any fluorescent molecule has two characteristics spectra: the excitation spectrum (the relative efficiency of different wavelengths of exciting radiation to cause fluorescence) and the emission spectrum (the relative intensity of radiation emitted at various wavelengths).

The shape of the excitation spectrum should be identical with that of the absorption spectrum of the molecule and independent of the wavelength at which fluorescence is measured. However, due to instrumental artifacts these two spectrums are seldom the same. A general rule of thumb is that the longest wavelength peak in the excitation spectrum is chosen for excitation of the sample. This minimizes the possible decomposition caused by the high energy, shorter wavelength radiation. Emission spectrum of a compound results from the re-emission of radiation absorbed by the molecule. Quantum efficiency and shape of the emission spectrum is independent of the wavelength of the exciting radiation (Lakowicz, 1999). Fluorescent compound used in this study was FITC (Fluorescein Isothiocyanate), which has excitation wavelength at 493nm and emission wavelength at 514 nm.

3.6.4 Fluorescence Measurements Fluorescence measurements can be broadly classified into two types, which are steady state and time resolved. Steady-state measurements are those performed with constant illumination and observation. This is the most common type of measurement. The sample is illuminated with a continuous beam of light, and the intensity or emission spectrum is recorded. Because of the nanosecond timescale of fluorescence when the sample is

exposed to the light, steady state is reached immediately. In case of time resolved measurements, the sample is exposed to a pulse of a light, where the pulse's width is typically shorter than the decay time of the sample. The intensity is recorded with a high –speed detection system (Lakowicz, 1999).In the present detection system, fluorescence measurements were done when steady state was reached.

3.6.5 Relation between Fluorescence Intensity and Concentration The basic equation, which relates fluorescence intensity to, the concentration of the compound, is following:

$$F = \phi I(1 - e)^{-\varepsilon bc} \quad (4)$$

Where ϕ is the quantum efficiency, I is the incident radiant power, ε is the molar abosrptivity, b is the path length and c is the molar concentration. On the basis of the above equation, there are three major factors other than concentration that affect the fluorescence intensity- quantum efficiency, intensity of radiation, and molar absorptivity (Lakowicz, 1999). The principle of fluorescence spectroscopy can be summarized as follows: An incident beam of radiation of a given wavelength is passed through a sample cuvette containing the fluor. A photomultiplier tube detects emitted radiation at 90° to the direction of the incident light beam and a monochromator is required to select wavelength of the emitted light (Sheehan, 2000) (Figure 9).

3.7 Fluorescence Based Assay for the Detection of Prions

Fluorescent dyes have many advantages as sensing probes to study protein-ligand interactions. Commonly, dyes are useful because they are sensitive to the conformation changes and changes in the surroundings (Stryer, 1968). This trait of extrinsic dyes can be exploited to sense the changes in the microenvironment. These transformations in dye can be seen as absorption characteristics or fluorescence emission spectra of a fluor. Some extrinsic dyes are nonfluorescent in aqueous (Polar) solution, but become fluorescent in a hydrophobic (Non polar) environment (Lopatin, 1971). A good example of this behavior is ANS (Anilinonaphthalene-1- Sulfonate) that has strong fluorescence in the hydrophobic regions of proteins where the unbound dye is non-fluorescent (Parker, Osterland, 1970). Another property of extrinsic dyes, which can be exploited in detecting the local environment changes, is their sensitivity to the pH change. An example of this type of dye is FITC (Fluorescein isothiocyante). Change in the fluorescence intensity of FITC due to pH change can be seen in (Figure10).

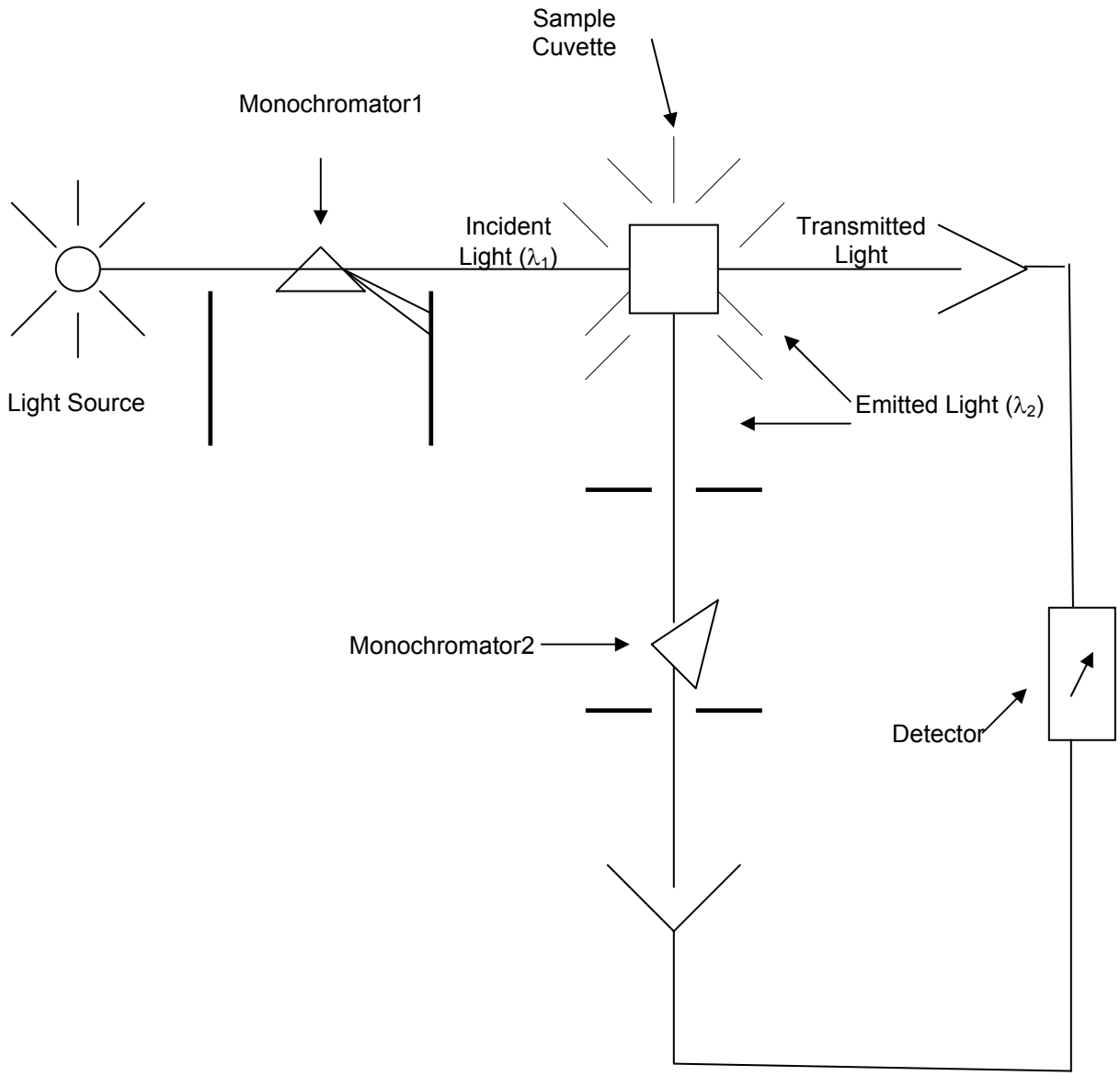

Figure 9: Redrawn schematic diagram of an ultra violet spectrofluorimeter. Light wavelength (λ_1) is absorbed by the sample in the cuvette. Emitted light of longer wavelength (λ_2) is passed through a monochromator and detected (Sheehan, 2000).

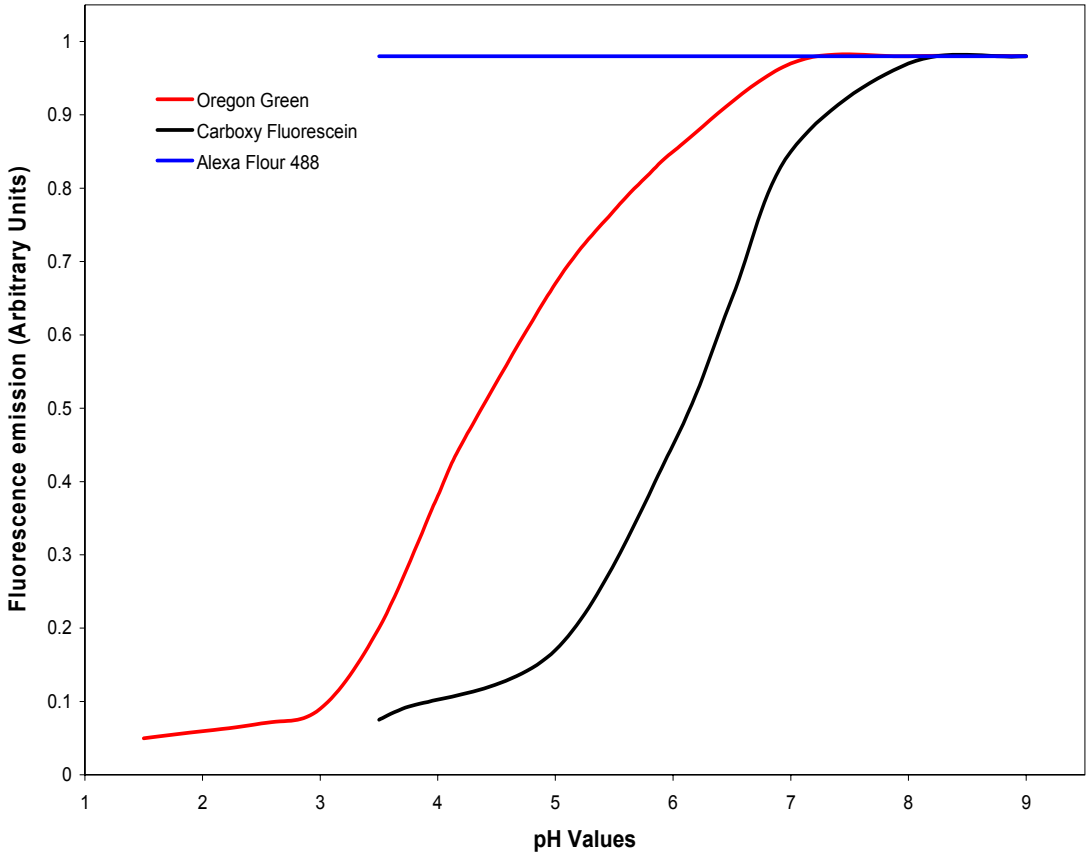

Figure 10: Redrawn comparison of pH-dependent fluorescence of the oregon green 488, carboxy-fluorescein and alexa fluor 488 fluorophores. Fluorescence intensities were measured for equal concentrations of the three dyes using excitation/emission at 490/520 nm (www.molecularprobes.com).

These effects (hydrophobicity or pH) will be used to design an assay based on the change in fluorescence of the dye bound to antibody. It can be predicted that following reactions will take place in the detection system—

$$Ab + D \Leftrightarrow (Ab * D) \quad (5)$$

$$(Ab * D) + D \Leftrightarrow (Ab * D2) \quad (6)$$

Where *Ab* is the antibody, *D* is a dye molecule attached with to a prion epitope, which binds to the antibody. *Ab*D* and $Ab*D_2$ are antibody dye complexes.

The fluorescence of the antibody dye complexes is different from the dye alone. Change in the fluorescence intensity of the dye upon binding to antibody can be attributed to the change in the local atmosphere (hydrophobicity or pH change) of the bound dye. It was assumed that two dye molecules can bound per antibody molecule, binding affinity of one or two dye molecules will be the same and the presence of second dye molecule will not effect the fluoresecence of antibody dye complex.

3.8 Detection System

On the basis of above mentioned model, change in the fluorescence intensity was utilized to detect the molecule of interest (prion). The principle of the system can be summarized as:

1. Anti-prion antibody was incubated with the dye attached to a prion epitope (QYQRES). The prion epitope bound specifically to the binding site of antibody.

2. Fluorescence of the sample was measured in the absence of prion.

3. In the presence of excess analyte molecule, the dye molecule was displaced from the binding site of antibody (Competitive Immuno Assay).

4. Fluorescence of the sample was measured again in the presence of prion.

5. Change in fluorescence (The dye was not in the close vicinity to the antibody so hydrophobicity or pH change should not affect the fluorescence of dye). Intensity was used to relate the presence and concentration of prion in the samples.

3.9 Schematic Diagram of the Biosensor

The detection system described above can be represented in a diagram. Following diagram represents the bio-sensing strategy used for detecting prion in foods (Figure 11):

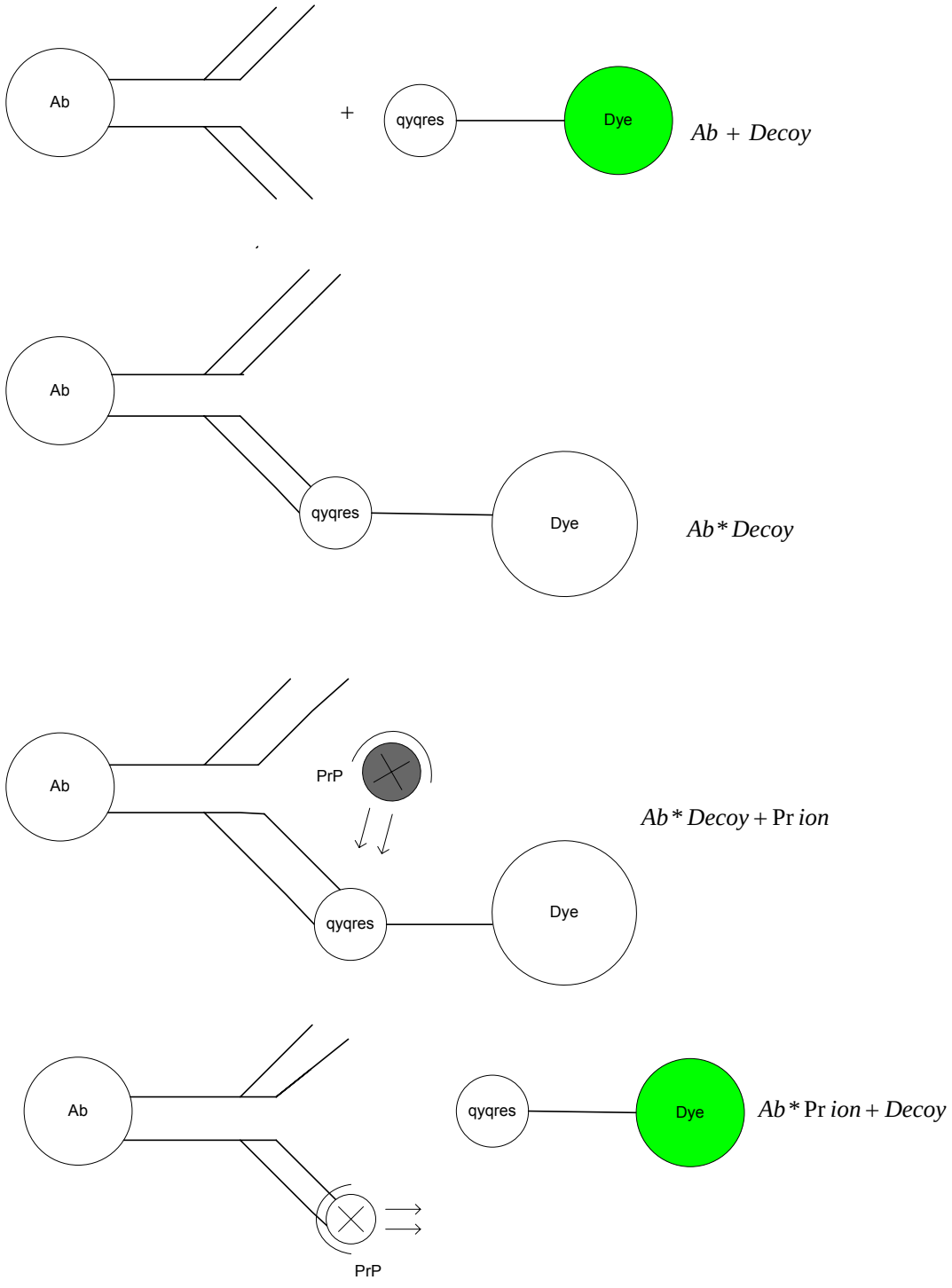

Figure 11: Biosensor scheme.

CHAPTER IV

MATERIALS AND METHODS

4.1 Materials

4.1.1 Antibody- Anti TSE monoclonal antibody derived from the cell line F99/97.6.1 was procured from VMRD, Inc. (Pullman, WA, USA).

4.1.2 Prion Protein- Lyophilized samples of prion peptides (prion protein, His Tag. Bovine, Recombinant E.coli) were obtained from BioSciences, Inc. (LaJolla CA, USA).

4.1.3 PrP epitope labeled FITC- Labeled Fluorescein (FITC-GABA-QYQRES) was custom made by Multiple Peptide Systems (San Diego, CA, USA).

4.1.4 Chemicals- Sodium phosphate, sodium azide, sodium dodecyl sulphate (SDS), Triton-x-100. These chemicals were used in buffer preparation; they are available at Sigma Chemicals (St. Louis, MO, USA). Protein A column from (Amersham Biosciences, NJ, USA).

4.1.5 Baby Formula- Good Start Brand Nestle (Los Angeles, CA, USA).

4.1.6 Gelatin- Unflavored gelatin powder manufactured by Knox (Glen View, IL, USA).

4.2 Reagent Preparation

4.2.1 Buffer Solution- 0.6 gm of monobasic sodium phosphate was added to 50 ml distilled water; 0.01% sodium azide was added to prevent microbial

contamination in the buffer. The solution was mixed well on a rotator for 10 minutes and the pH was adjusted to 7 ±0.01 using HCl/ NaOH.

4.2.2 Decoy Solution- Stock solution of decoy (FITC dye and prion epitope) was prepared by dissolving the dye into the buffer; serial dilutions were done to obtain the desired concentration of decoy in the final samples. The pH of the decoy was checked frequently to ensure it is fixed at 7± 0.01.

4.2.3 Antibody- The antibody was purified using affinity chromatography on an r protein A column. 2mg/ml of bovine serum albumin (BSA) was added to the antibody for stabilization during storage. Dilution of antibody was done using phosphate buffer. The molecular weight of antibody used for calculations was 150,000 Daltons.

4.2.4 Gelatin Solution- Gelatin solution was prepared by dissolving powdered gelatin and SDS (Sodium Dodecyl Sulphate) in 0.1M sodium phosphate buffer. The solution was kept in a water bath at (35-40ºC) and shook intermittently to prepare a clear solution of gelatin with no lumps. The pH of the solution was adjusted by adding very small amounts of acid (0.1M HCl) or base (0.1M NaOH). Depending on the final concentration of gelatin in the samples, the stock concentration was adjusted. SDS concentration was fixed at 0.3mg/ml in the gelatin samples. Fresh solution of gelatin with SDS was prepared for all the experiments to avoid any kind of microbial contamination.

4.2.5 Baby Formula Solution- Stock solution of baby formula was prepared by adding 29 gm of dry formula in 100 ml of 0.1M phosphate buffer. The

temperature of the buffer was adjusted to 35-40ºC by keeping it in a water bath. The solution was kept on a tumbler for 3-4 hours to achieve a homogenous solution. Final pH was adjusted to 7±0.01 by adding very small amounts 0.1M HCl or 0.1M NaOH. Fresh stock of baby formula solution was prepared for all the experiments to avoid microbial contamination. Serial dilutions of baby formula were done using phosphate buffer only. Extent of dilution was 10 times, 20 times, 40 times, and 5000 times for different experiments. 0.454 mg/ml of Triton-X 100 was added to the final baby formula samples.

4.3 Fluorescence Measurement

Changes in the fluorescence intensity were measured by testing the following samples:

4.3.1 Control Sample- Buffer (0.1 M Sodium Phosphate) and labeled analog of target analyte (decoy).

4.3.2 Antibody Sample- Buffer, decoy and anti-prion antibody.

4.3.3 Prion Sample- Buffer, decoy, anti-prion antibody and prion.

On the basis of previously mentioned hypothesis, increase in the fluorescence signal of antibody sample from control sample was due to pH change or to hydrophobicity. The prion sample intensity was close to the control value since the prion will displace the entire bound decoy from the antibody (Competitive Immuno Assay). Differences in intensity were used to calculate the concentration of prion in the sample.

4.4 Testing Procedure

Anti-prion antibody diluted in buffer (0.1M Sodium phosphate) was used for competitive immunoassay. Labeled analog of target analyte (decoy) (FITC-GABA-QYQRES) was diluted using the same buffer.

A control sample was prepared by adding decoy and buffer. Antibody sample was made by adding decoy and antibody in 1:1 ratio and adding buffer to make up the rest of the volume.

All the samples were covered with aluminum foil to prevent photo bleaching of the dye. The samples were placed on a rotator for 4 hours for the reaction to reach equilibrium.

After the reaction reached equilibrium, the fluorescence of control and antibody samples was measured using a spectrometer (Photon Technology International. Lawerenceville, NJ, USA).

Once the decoy was bound to the antibody-binding site, prion was added in an increasing ratio to decoy for different samples. The samples were kept on a rotator for 4 hours to reach equilibrium.

Fluorescence of the prion samples was measured again after the reaction reached equilibrium.

Differences in fluorescence intensities of decoy before and after addition of the target analyte were used to detect the presence of prion in the sample.

4.5 Optical Setup

Experimental data was collected using a fluorescence spectrometer (Photon Technology International, Lawerenceville, NJ, USA) that has PTI fluorescence system hardware and Felix for Windows software. Excitation source for spectrometer was an Argon laser (488nm) from Spectra Physics, (Houston, TX, USA). 500nm cutoff filter was also placed at the exit slit of the fluorometer. Square quartz cuvets Fisher Scientific, Pittsburgh, (PA, USA) size 10×10mm were used for testing the samples in the spectrometer.

Experiments were done for different concentrations and fixed ratio of antibody and decoy. Prion peptides were added at different concentrations to find the optimum range in which maximum change in fluorescence can be obtained. Most of the initial testing was done in phosphate buffer until the range of conditions that work well for the detection of prion was found. Similar experiments were done in food products to examine prion displacement. The experimental data was used to develop a mathematical model that can be used to determine the equilibrium constant for antibody decoy reaction.

All the experiments were done in triplicates and statistical analysis was done using statistical software SPSS (Version 11.0 for Windows).

CHAPTER V
RESULTS AND DISCUSSION

5.1 Effect of Environmental Changes on Dye Fluorescence

Generally extrinsic dyes are sensitive to the structural changes and change in their local environment. These changes can be used to study the microenvironment (Lopatin, 1971). Some dyes like ANS are sensitive to the polarity of the solution; ANS strongly fluoresces when bound to the hydrophobic regions of the protein while unbound dye is non-fluorescent (Lopatin, 1971). Another property of extrinsic dyes is their sensitivity to the changes in their local environment pH. A good example is fluorescein.

Fluorescein Isothiocyanante (FITC) is sensitive to the pH of the solvent and Figure (12) shows the dependence of FITC on the pH. As the pH of the buffer was increased from 5 to 9 the fluorescence intensity of the dye also increased to a maximum value. At pH 10 the value of Intensity decreased to a steady level. All the intensity values were significantly different at $p<0.05$.

Emission of the fluorescence intensity of FITC is also dependent on the polarity of the environment. Figure (13) represents the affect of different solvents on the fluorescence intensity. The maximum intensity was observed in the polar solvents like 0.1 M phosphate buffer; hydrophobic solvent decreased the intensity of FITC. Intensity values are significantly different at $p<0.05$. These properties of FITC were exploited in probing changes in the microenvironment of antibody after antigen binding. The increase in fluorescence intensity of antibody

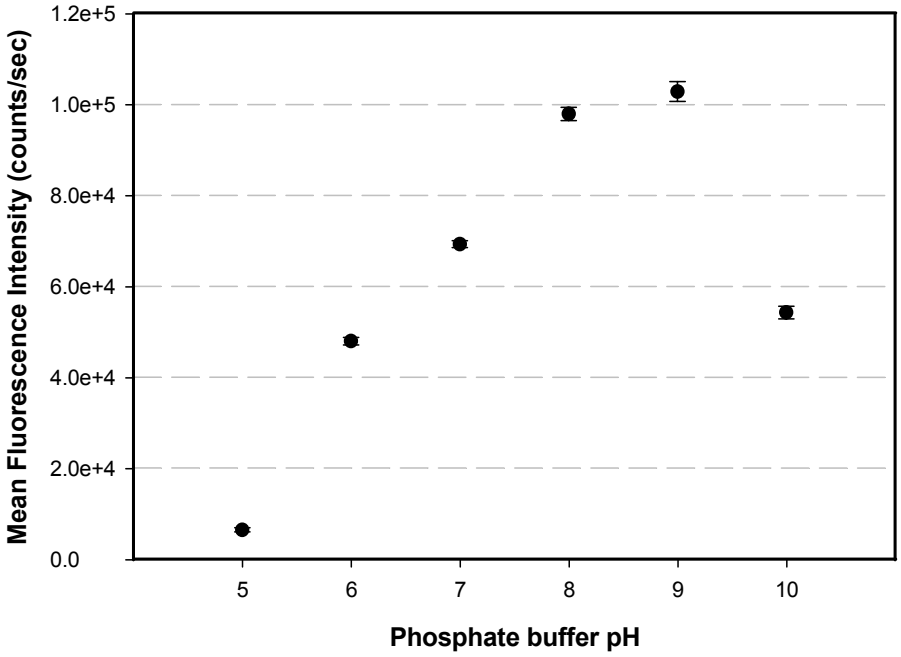

Figure 12: pH dependence of decoy in 0.1 M sodium phosphate buffer. Emission peak was at 514 nm. Decoy concentration was 4 nM. Data analysis (Appendix A-1).

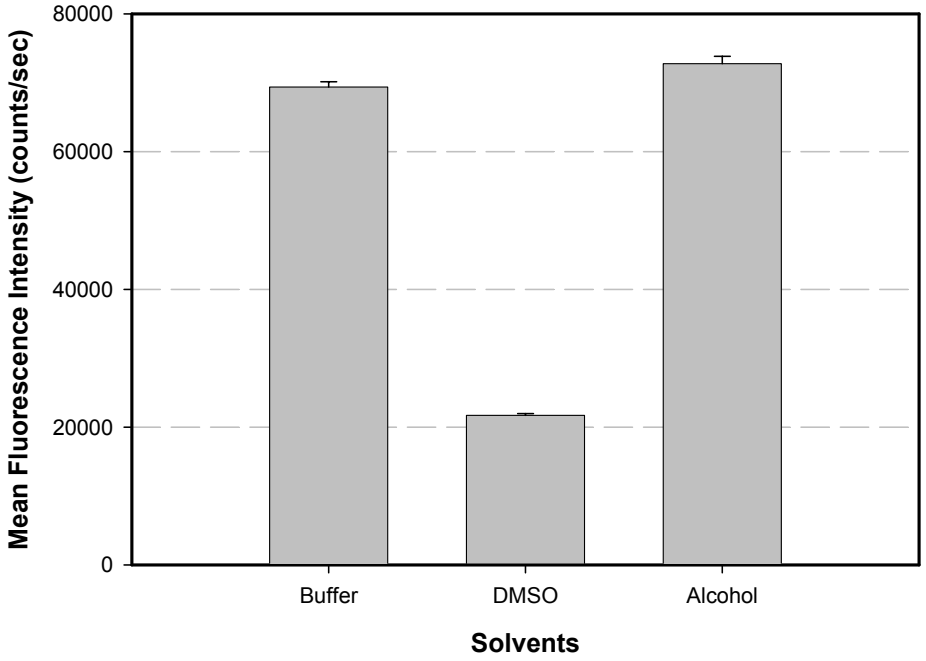

Figure 13: Effect of the polarity of solvent on dye fluorescence. Emission peak was at 514 nm. Data analysis (Appendix A-2).

samples can be based upon the following hypothesis: "*After the dye is bound to the antibody there is a change in the local environment of the dye that leads to the measurable change in fluorescence intensity of the dye*."

The change in the fluorescence can be attributed to the pH change or the hydrophobicity. In Figure (13), the fluorescence of the dye decreased in the more hydrophobic (intensity value is less in DMSO than ethanol) environment thus an increase in the fluorescence due to hydrophobicity is less likely. Hence, the change in the intensity can be attributed to the pH change.

5.2 Binding Studies of Antibody to Decoy

From Figure (12) and (13) change in the fluorescence of the dye was observed due to a change in the surrounding environment of the fluorophore. An increase in the fluorescence upon binding of the decoy (dye and prion epitope) to the antibody is due to a change in the pH. From Figure (12), the maximum change in the intensity was observed between pH6 and 8 the, dynamic range of pH change.

The maximum difference between the control and the antibody sample was observed at pH7 Figure (14). This may be due to the fact that in the control sample at pH 7 there is no change in the local pH of the decoy, but in the case of the antibody sample at pH 7 the local pH around the decoy advances towards 9 upon binding with the antibody. The differences were significant at $p<0.05$.

5.2.1. Equilibrium Studies To ensure that the effect observed upon the binding of decoy with antibody is stable and specific, more experiments were done to

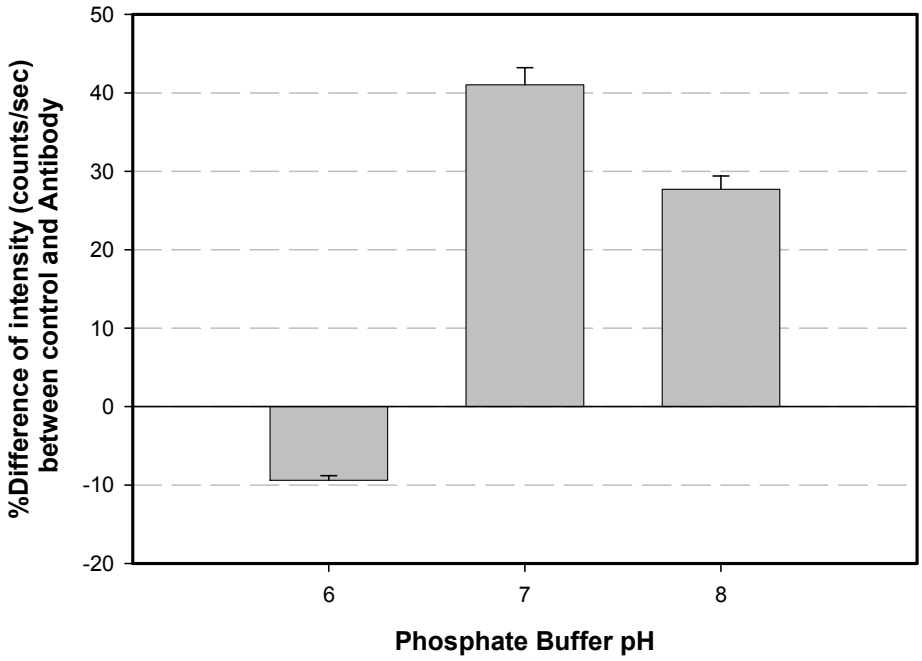

Figure 14: Effect of buffer pH on the % difference of the intensity between the control and antibody samples. Peak was measured at 514 nm. Decoy concentration was 4 nM and antibody was added in 1:1to decoy. Data analysis (Appendix A-3).

determine the equilibrium conditions. Figure (15) illustrates that the reaction reaches equilibrium in less than 4 hours and the change in the intensity was specific for antibody and the decoy. From Figure (16) it is evident that the same effect was not observed when Bovine Serum Albumin (BSA) was used instead of the antibody. All the values were significantly different at $p<0.05$.

Hence, the change in fluorescence of the decoy upon binding with the anti-TSE antibody is specific and the reaction reaches equilibrium in 4 hours. From this point onwards the incubation time for all the further experiments was 4 hours at room temperature.

5.2.2 Determination of Equilibrium Constant The equilibrium constant K, for the reaction between the antibody and the decoy was determined using Law of Mass action.

Experimental data was obtained keeping decoy concentration constant at 10nM and antibody concentration was varied from 0 to 110nM. Fluorescence intensity of the decoy was measured and the data was analyzed using the following model to obtain the *K* value:

$$Ab + D \Leftrightarrow Ab * D \tag{7}$$

Equation (8) represents a general equilibrium reaction between antibody and decoy. The expression for the equilibrium constant on the basis of the mass action model was given as:

$$K = \frac{[(Ab * D)]}{[Ab][D]} \tag{8}$$

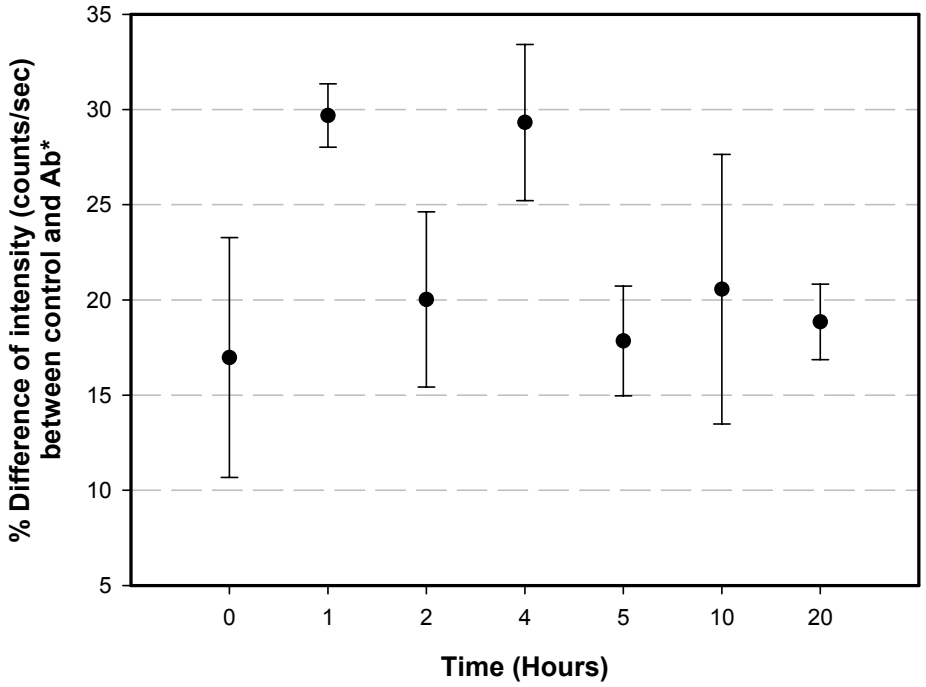

Figure 15: Equilibrium studies for antibody-decoy binding. % Difference represents intensity difference between control and antibody samples. Decoy concentration was 4 nM and antibody was added in 1:1to decoy. Data analysis (Appendix A-4). Ab*-Antibody

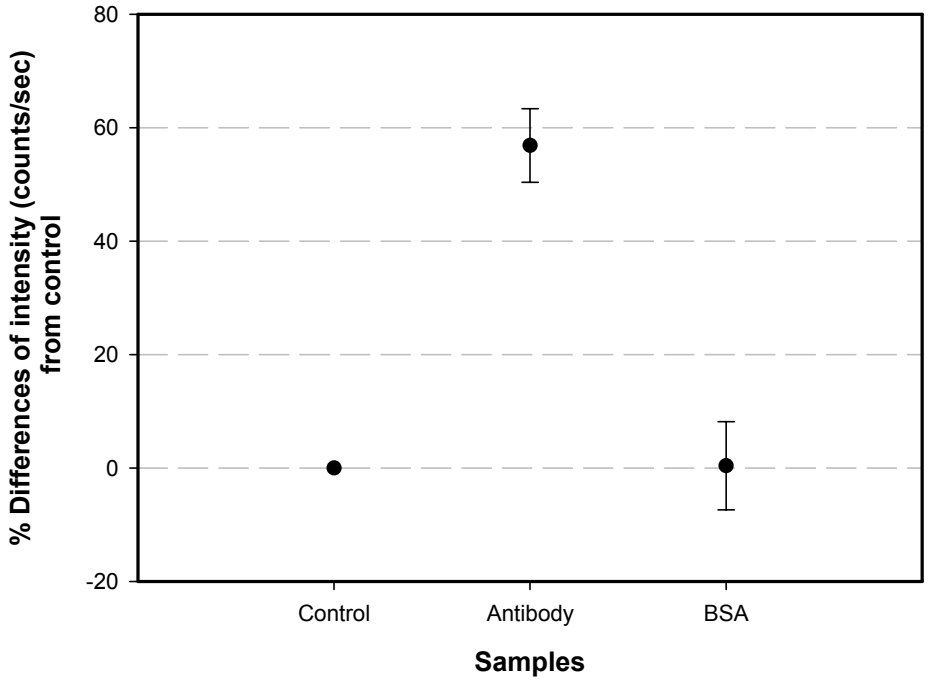

Figure 16: Specificity of the antibody-decoy binding. Other protein (BSA) does not exhibit similar interactions. Decoy concentration was 4 nM and antibody was added in 1:1 to decoy. Data analysis (Appendix A-5).

After applying mass balance on the total concentration of antibody and decoy following equations were obtained:

$$Ab_T = Ab_O + [(Ab * D)] \tag{9}$$

$$D_T = D_O + [(Ab * D)] \tag{10}$$

Fluorescence intensity of control and antibody solutions was related to the concentration of the free and bound decoy in the solution.

$$I = a[D_O] + b[(Ab * D)] \tag{11}$$

$$I_O = a[D_T] \tag{12}$$

Equation (11) was rearranged and

$$I = a[D_T - (Ab * D)] + [(Ab * D)] \tag{13}$$

Equation (8) was also rearranged using equations (10) and (11):

$$K = \frac{[(Ab * D)]}{[Ab_T - (Ab * D)][D_T - (Ab * D)]} \tag{14}$$

Using equations (13) and (14) an expression was derived relating K and intensity of the solutions.

$$\frac{I}{I_O} = 1 + \frac{(b/a - 1)}{D_T} \frac{Ab_T + D_T K + 1 - \sqrt{Ab_T{}^2 K^2 + D_T{}^2 + 1 - 2Ab_T D_T K^2 + 2Ab_T K + 2D_T K}}{2K} \tag{15}$$

Equation (16) can be used to estimate equilibrium constant. The value of b/a was estimated as the ratio of intensities at zero and maximum antibody concentration. It was assumed that binding of one decoy does not affect the binding affinity of the other decoy to bind. The value of the equilibrium constant was close to $1X10^9$ $Mole^{-1}$ at room temperature. Figure (17) presents the binding

affinity curve of the antibody at a fixed decoy concentration. The optimal operational range for the sensor is at low concentration values (<20 nM) before saturation effect is observed.

5.3 Prion Studies in Buffer

To study the displacement of decoy with prion, samples containing antibody, decoy, buffer, and prion were tested against the control (buffer and decoy) and antibody samples (buffer, antibody and buffer). Intensity was measured for all the samples and the difference in intensity was used to detect the presence of prion. Table (3) represents the prion displacement. As the prion concentration increases the intensity of the prion sample should approach that of the control value. It is evident that at low concentration of prion (10 nM), the sample intensity was close to the control, but at 20 nM prion concentration there was an unexpected increase in the prion samples.

The possible reason for this behavior was the long incubation time (20 hours) of the prion samples, which led to prion aggregation, since prions are known to aggregate at high concentrations (Gasset et al., 1993; Safar et al., 1994). An increase in the intensity of the control samples with prion can also be attributed to prion aggregation (Table 4). Intensity values for prion controls (20, 60, and 90 nM) were significantly different from 0 nM prion at $p<0.05$.

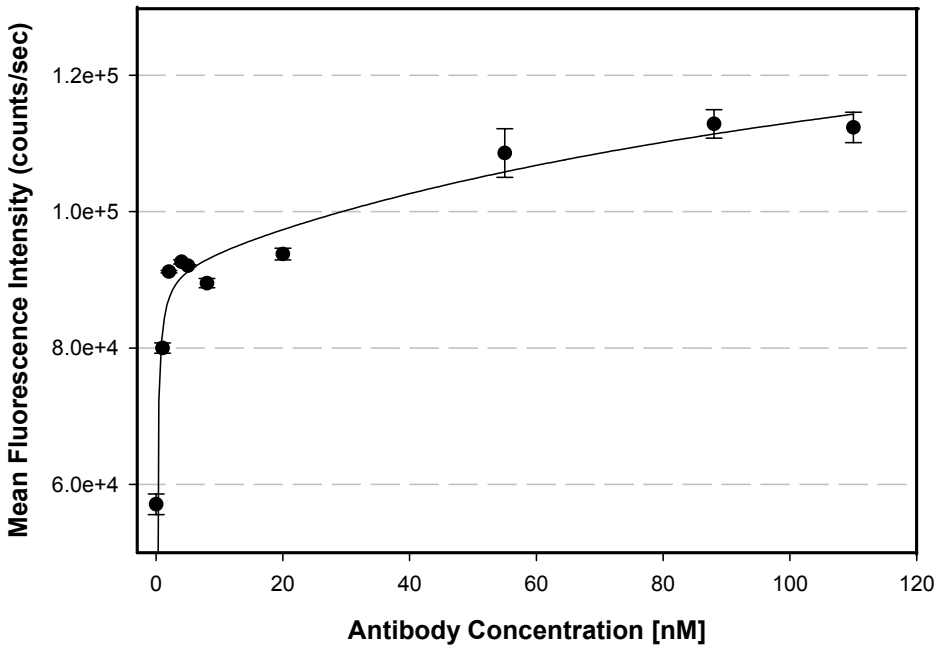

Figure 17: Binding affinity curve. Antibody concentration was varied from 0 to 110 nM and decoy concentration was fixed at 10 nM. At high concentrations of antibody (>20 nM) saturation effect was observed. Data analysis (AppendixA-6).

Table 3:

Decoy displacement by prion. Concentration of decoy 10 nM, antibody concentration 10 nM, prion was added in the increasing ratio to decoy. Incubation time for all the samples was 20 hours. Data Analysis (Appendix A-7).

Sample	Prion Concentration [nM]	Mean Intensities (Counts/sec)	Intensity difference from no Prion sample
Control	-	40132.73	-
Antibody sample	0	63613.30	0.00%
Antibody and prion	10	56912.20	-10.53%
Antibody and prion	20	74020.00	16.36%
Antibody and prion	60	81498.63	28.11%
Antibody and prion	90	108978.67	71.31%

Control = Buffer + Decoy, Antibody = Buffer + Decoy + Antibody, Antibody and prion = Buffer + Decoy + prion

Table 4:

Mean intensity values of prion controls (buffer, decoy and prion). Incubation time for samples were 20 hours. Data Analysis (Appendix A-8).

Sample	Mean Intensities (Counts/sec)	Difference between no prion sample and control
Control and 0nM prion	40132.73	0.00%
Control and 10nM prion	43974.30	9.57%
Control and 20nM prion	45185.03	12.58%
Control and 60nM prion	49166.63	22.51%
Control and 90nM prion	68122.73	69.74%

Control = Buffer + Decoy, Control and prion = Buffer + Decoy + prion

To reduce the risk of aggregation a kinetic study was done to determine the optimum incubation time for decoy displacement by the prion. From Table (3) and (4) it was evident that prion concentration higher than 50 nM leads to aggregation. Therefore the kinetic study was done at 60nM of prion. Figure (18) explains the results of the kinetic study. The maximum difference between prion and no prion samples was observed at 4 hours. Hence 4 hours are sufficient for the decoy to be displaced by prion. As the time increased, the intensity values for the prion sample increased again. From the above experiments it was evident that high concentration prion samples had an unusual behavior and measures should be taken to avoid prion aggregation in solution. More kinetic studies showed similar behavior for the prion samples.

From this point onwards all the prion displacement experiments were conducted at low concentrations and incubation time for all the samples was 4 hours. Figure (19) represents another decoy displacement study where incubation conditions were similar to the previous findings. The minimum detection concentration of prion was 8 nM at $p<0.05$. Samples from 8 nM onwards were significantly different from each other using LSD at $p<0.05$. The maximum detectable concentration of prion was 20nM in this experiment. There was no significant difference between 20 and 40 nM samples since most of the decoy was replaced by prion at 20 nM. Prion aggregation was not observed after 4 hours of incubation.

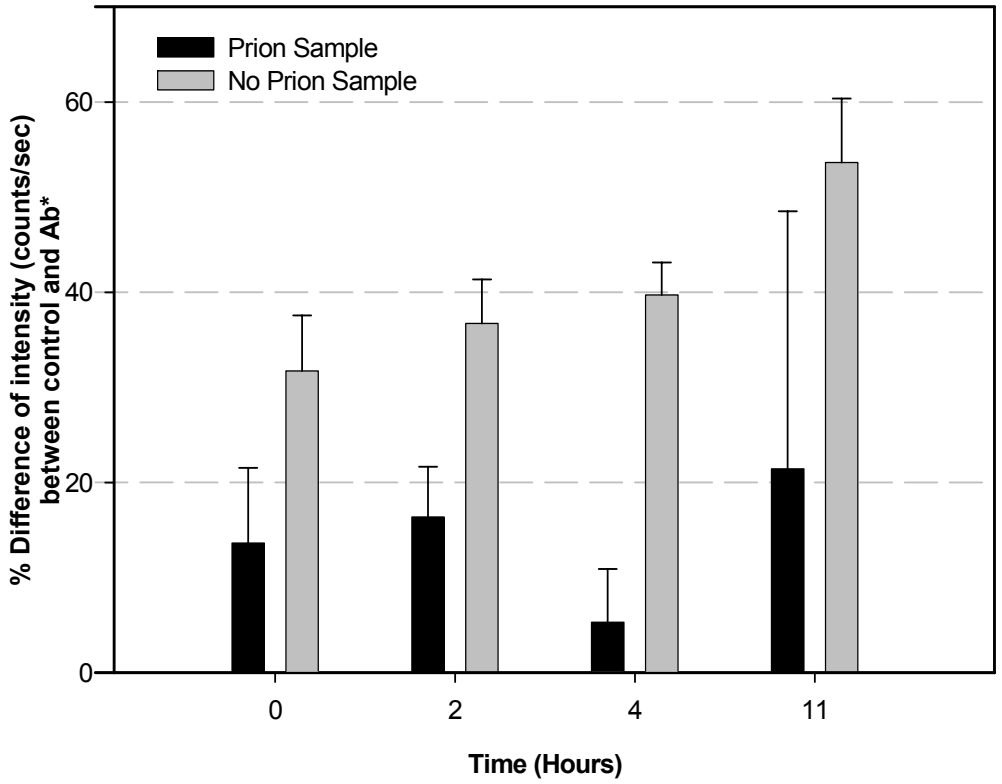

Figure 18: Kinetic study for decoy displacement by prion. Percentage difference indicates the intensity difference from control sample. Decoy concentration was 10 nM, antibody concentration was 10 nM and prion concentration was 60 nM. Data analysis (Appendix A-9).

Ab*-Antibody

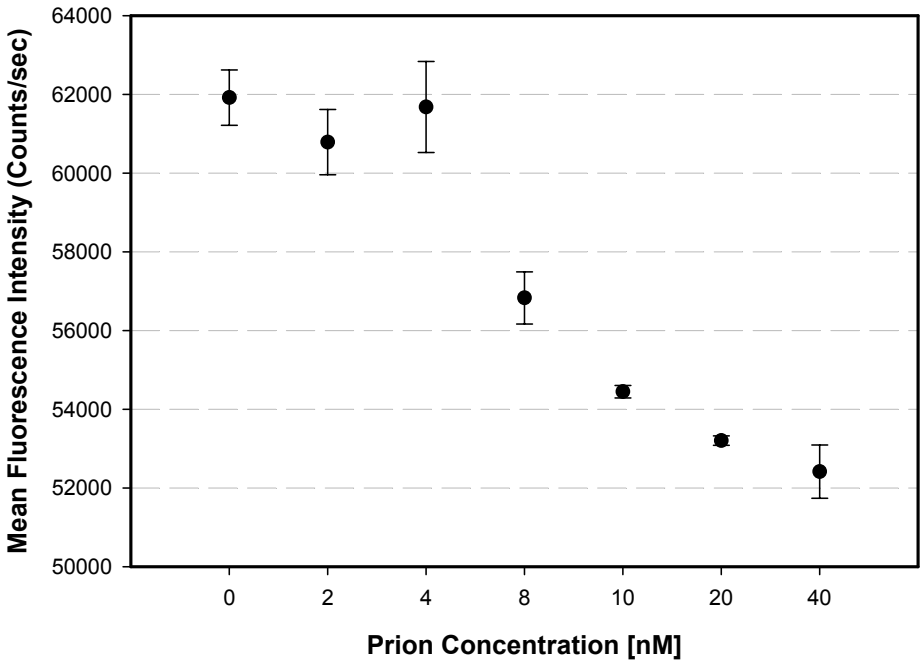

Figure 19: Decoy displacement plot in 0.1M phosphate buffer. Decoy concentration 4 nM, antibody concentration 4 nM, and prion was added in the increasing ratio to the decoy. Data analysis (Appendix A-10).

On the basis of this experiment it was concluded that sensitivity of detection is between 8 nM and 20 nM of prion; after that saturation effect was observed. Prion was always added in an increasing ratio to decoy therefore to probe the lower detection limit of prion detection. Concentration of decoy was reduced from 4 nM to 1 nM. The lowest detectable concentration of prion in this experiment was 200 pM.

Figure (20) shows the result of decoy displacement at 1nM decoy concentration. All the prion samples other than 5 nM were significantly different ($p<0.05$) from the no prion sample, which could be an experimental anomaly. Samples were significantly different from each other. There was a decrease in the fluorescence of the no prion sample compared to the control sample, which was not the case at 4 nM and 10 nM decoy concentrations. This behavior was consistent. It was hypothesized that at 1nM decoy concentration the binding affinity of antibody alters or the time taken to reach equilibrium conditions was different than the higher concentrations of decoy. Further investigation is needed to determine the equilibrium conditions at very low decoy concentrations.

Prion detection experiments performed in phosphate buffer at the different concentrations of decoy revealed that prion could be detected using this biosensor. Detection limit was as low as 8 nM at 4 nM decoy concentration and 200 pM at 1 nM decoy concentration. Since all the initial conditions for prion detection were determined in phosphate buffer, experiments were initiated to implement the same technique in food products.

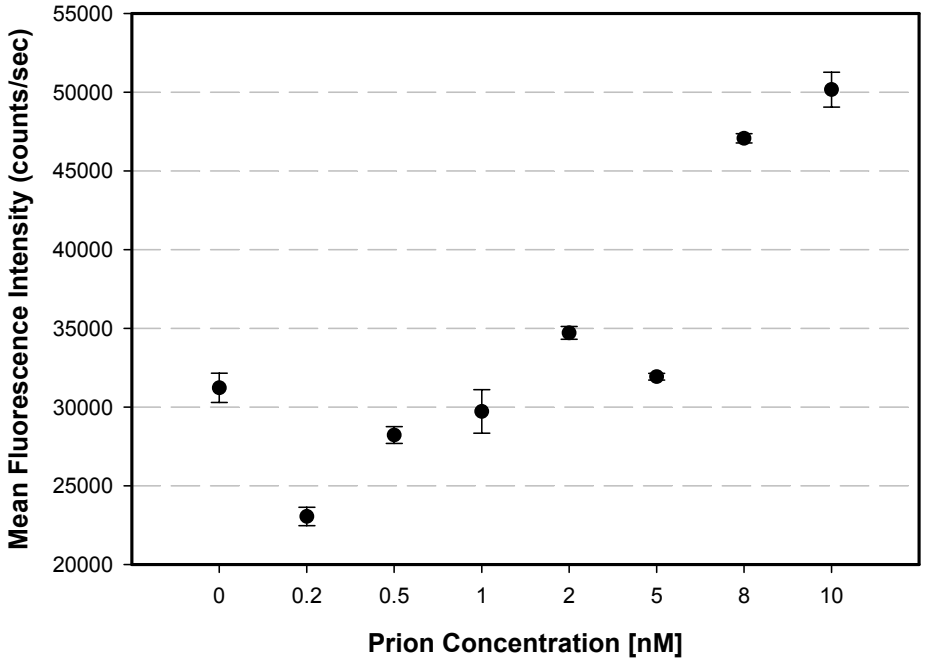

Figure 20: Decoy displacement plot at 1 nM decoy concentration. Antibody concentration was 1 nM and prion was added in the increasing ratio to the decoy. Lowest detectable prion concentration was 200 pM. Data analysis (Appendix A-11).

5.4 Prion Detection in Gelatin Solutions

Gelatin is a complex protein and its interactions with prion or decoy are not known. An initial prion detection experiment was done in low gelatin concentration to minimize the interaction of gelatin with other bio-molecules in the system. Figure (21) illustrates prion detection in 0.01 mg/ml gelatin solution. The lowest detectable concentration was 4nM at $p<0.05$ from no prion sample. The 4, 8 and 10 nM samples were significantly different from each other at $p<0.05$. 2 nM sample was not significantly different from the 0 nM prion sample.

Figure (22) shows the decoy displacement in 0.4 mg/ml gelatin solution. In this case the lowest detectable concentration of prion was 2nM but, different prion concentration samples were not significantly different from each other. Only the 2, 8 and 10 nM samples can be differentiated from each other. Results from this experiment indicate that the dynamic range of detection is low in 0.4 mg/ml gelatin solutions as compared to 0.01 mg/ml gelatin.

A plausible explanation for this behavior could be that gelatin interferes with the binding of decoy to the antibody or with the displacement of decoy by prion. To minimize the interactions of free groups on the side chains of gelatin with other molecules in solution, a small amount of surfactant was added to the gelatin samples. Several classes of polymers interact strongly with surfactants; these interactions are electrostatic or hydrophobic in nature (Griffiths et al., 1997).

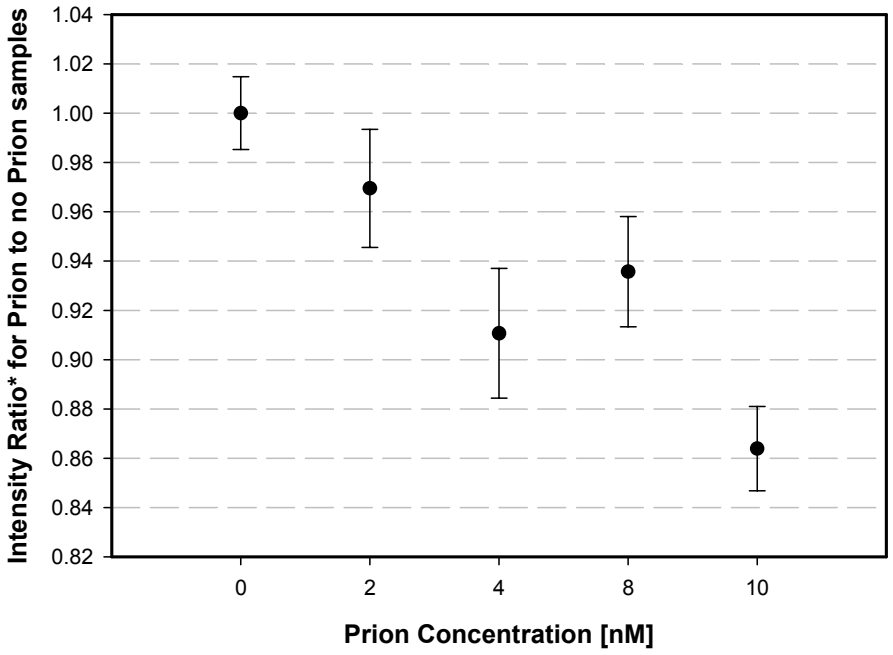

Figure 21: Prion detection curve in 0.01 mg/ml gelatin. Decoy concentration was 4 nM , antibody concentration was 4 nM, and prion was added in the increasing ratio to decoy. Data analysis (Appendix A-12).

*Intensity Ratio** is defined as the ratio of mean fluorescence intensities of prion samples to the no prion sample/antibody sample.

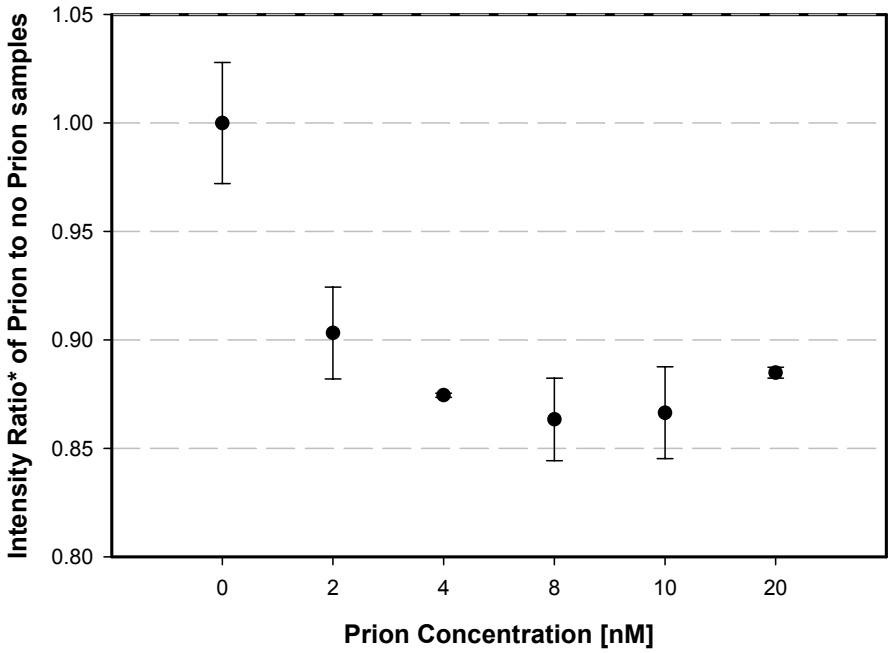

Figure 22: Prion detection curve in 0.4 mg/ml gelatin. Decoy concentration was 4 nM, antibody 4 nM; prion was added in the increasing ratio to decoy. Lowest detectable prion concentration was 2 nM. Data analysis (Appendix A-13).

*Intensity Ratio** is defined as ratio of mean fluorescence intensities of prion samples to the no prion sample/antibody sample.

Therefore weakly charged polyampholytes such as gelatin also form complexes with anionic surfactants, which stabilize the structure and free groups on the side chains of gelatin (Griffiths et al., 1997).

The low dynamic range of prion detection in 0.4 mg/ml gelatin may be due to small differences between gelatin control and gelatin antibody samples Figure (22). Hence, to improve sensitivity of detection it is imperative to increase the differences between gelatin control and gelatin antibody samples. SDS (0.3mg/ml) was added to the samples and Table (5) shows the results. Addition of SDS increased the difference between gelatin control and gelatin antibody samples from 11% to 20%. The differences between average intensities were significant at $p<0.05$.

On the basis this result, prion displacement experiment was repeated in 0.4 mg/ml gelatin with 0.3mg/ml SDS. Addition of SDS to the gelatin samples improved the sensitivity of detection as well as the detection limit Figure (23). The lowest detectable prion concentration was 0.5 nM and the maximum detectable concentration was 5 nM. The 1nM prion sample was significantly different from 0.5 and 0.8 nM prion sample but, it was not significantly different from the 2, 5nM samples. This could be an experimental anomaly. The addition of SDS to the samples improved the dynamic range of detection from 0.5 to 5nM at $p<0.05$ Figure (23).

Table 5:

Effect of SDS on the differences between gelatin control and gelatin antibody samples. Data Analysis (Appendix A-14).

Sample No.	Tested Sample	Average Intensity (Counts/sec)	% Difference of intensity	Coefficient of variation
1	Control	104436.00	-	-
2	Antibody sample	129755.00	24.24	
3	Gelatin control	133127.67		2.55%
4	Gelatin Antibody sample	147980.67	11.16	3.23%
5	Gelatin SDS control	99916.47	-	1.08%
6	Gelatin SDS antibody sample	119617.33	19.72	1.87%

Control = Buffer + Decoy, Antibody Sample = Buffer + Decoy + Antibody, Gelatin control = Buffer + Decoy + Gelatin, Gelatin antibody = Buffer + Decoy + Gelatin + Antibody, Gelatin SDS control = Buffer + Decoy + Gelatin + SDS, Gelatin SDS antibody sample = Buffer + Decoy + SDS + Gelatin + Antibody.

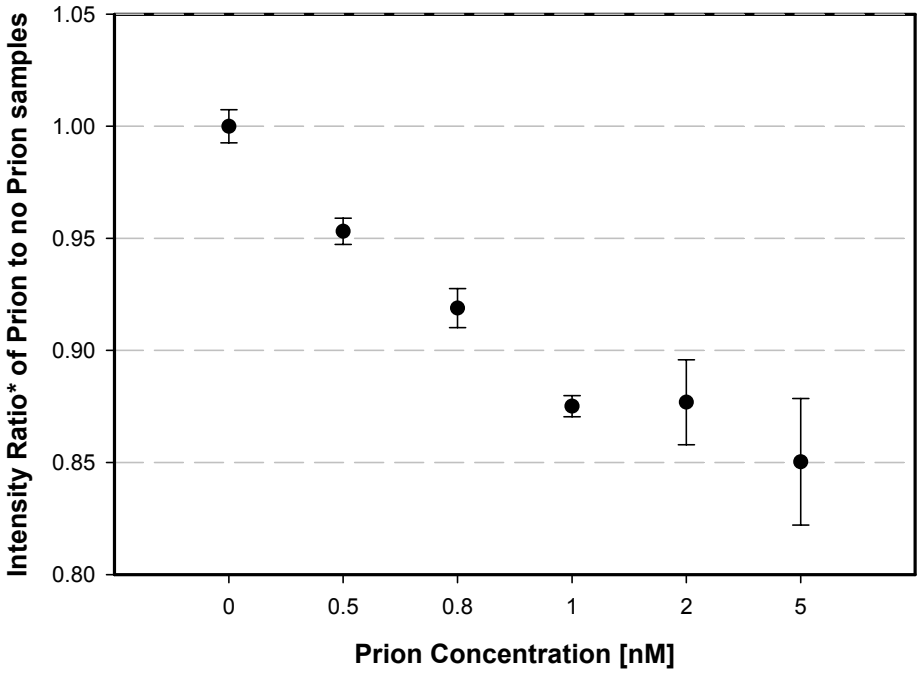

Figure 23: Prion detection curve in 0.4 mg/ml gelatin with 0.3 mg/ml SDS. Decoy concentration was 4 nM, antibody 4 nM; prion was added in the increasing ratio to decoy. Lowest detectable prion concentration was 0.5nM. Data analysis (Appendix A-15).

*Intensity Ratio** is defined as the ratio of mean fluorescence intensities of prion samples to the no prion sample/antibody sample.

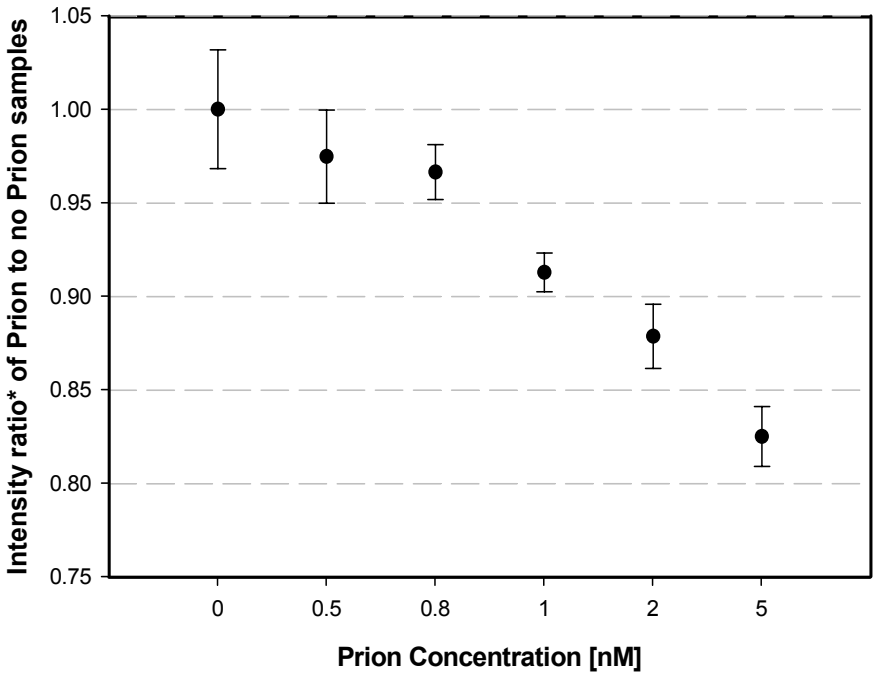

Figure 24: Prion detection curve in 1.0 mg/ml gelatin with 0.3 mg/ml SDS. Decoy concentration was 4 nM, antibody 4 nM; prion was added in the increasing ratio to decoy. Lowest detectable prion concentration was 1.0 nM. Data analysis (Appendix A-16).

*Intensity Ratio** is defined as the ratio of mean fluorescence intensities of prion samples to the no prion sample/antibody sample.

Another experiment of prion detection with 1 mg/ml of gelatin and 0.3 mg/ml of SDS was performed; Figure (24) illustrates the effect of high gelatin concentration on prion detection. In 1 mg/ml gelatin solution the lowest detectable concentration of prion was 1nM at $p<0.05$. Samples with 1, 2 and 5nM prion concentration were significantly different from the no prion samples at $p<0.05$. It was evident that increasing the concentration of gelatin lowered the detection limit of the biosensor. High gelatin concentrations may adversely affect the sensitivity of prion detection. More investigation is required in this area.

An odd problem was encountered when 2 mg/ml gelatin experiments were conducted. The response from the antibody was not the same as compared to the previous experiments. All the experimental variables like concentration of the solutions, pH of the buffer, and optical system were checked to ensure the consistency of the experimental setup. After eliminating all the possible variables it was concluded that the antibody was not in the same state as it was in the previous experiments. A SDS-PAGE gel done run to find the status of antibody and it was clear from the results that the antibody was not aggregated and it was in good condition. Purification of antibody was done using a protein- A column and the concentration of the antibody was found to be 0.5 mg/ml instead of expected 1mg/ml Figure (25).

Table (6) shows the effect of antibody concentration on the difference between the antibody and control samples. The difference between control and antibody sample increased as the ratio (antibody: decoy) was higher.

Table 6:

Effect of antibody concentration on the difference between control and antibody sample. Decoy concentration was 4 nM. Data Analysis (Appendix A-17).

Sample	Antibody:Decoy	Antibody Concentration [nM]	Mean Intensities (Counts/sec)	% Difference
Control (Buffer and Decoy)	0	0	116385.00	0
Antibody1 (Buffer+Antibody+Decoy)	1	2.0	124537.30	7.00
Antibody2 (Buffer+Antibody+Decoy	1.6	3.2	125749.70	8.05
Antibody3 (Buffer+Antibody+Decoy)	2	4.0	135077.70	16.06

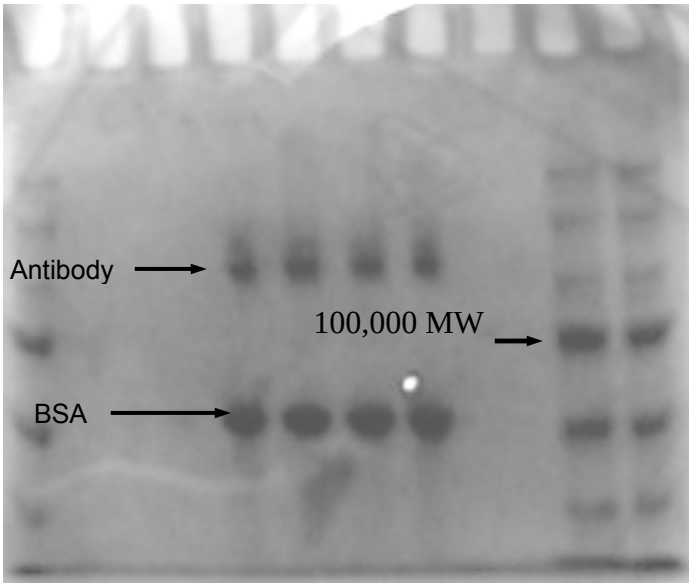

Figure 25: Sodium dodecyl sulphate poly acyrilamide gel (SDS-PAGE) picture. Different bands represent the proteins present in the solution. Extreme right bands are standard molecular weight proteins.

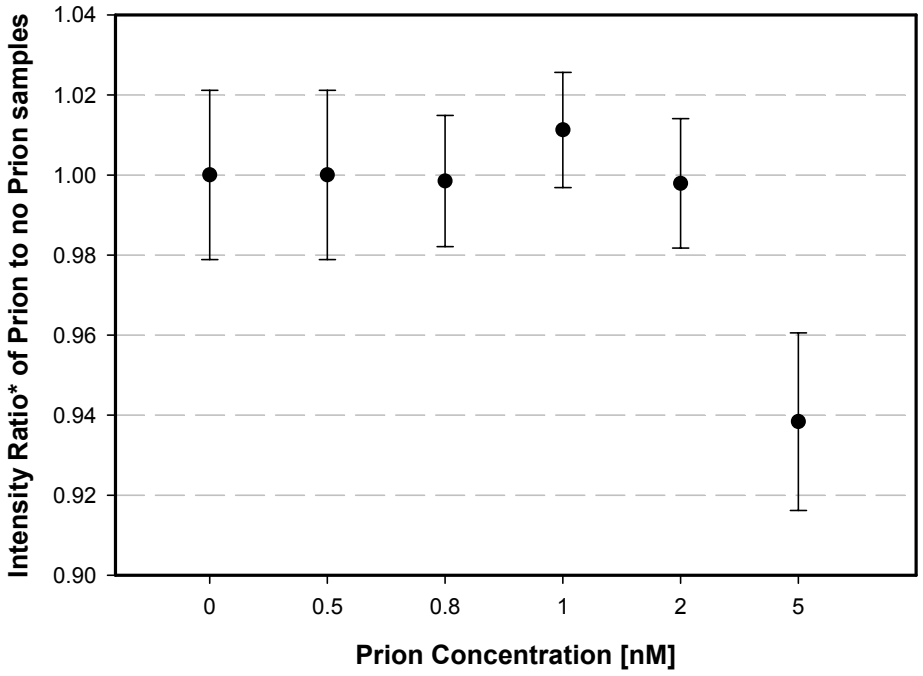

Figure 26: Prion detection curve in 2.0 mg/ml gelatin with 0.3 mg/ml SDS. Decoy concentration was 4 nM, antibody 4 nM; prion was added in the increasing ratio to decoy. Lowest detectable prion concentration was 2.0 nM. Data analysis (Appendix A-18).

*Intensity Ratio** is defined as the ratio of mean fluorescence intensities of prion samples to the no prion sample/antibody sample.

The difference between mean intensities was significantly different at $p<0.05$. After appropriate adjustment in the antibody concentration similar response upon decoy binding from antibody was obtained. Figure (26) illustrates the decoy displacement study in 2 mg/ml gelatin and 0.3mg/ml SDS. The Minimum detectable concentration of prion was2 nM at $p<0.05$. There was no significant difference among the 0.5, 0.8 and 1.0 nM samples and no prion sample. The 2 nM and 5 nM prion samples were significantly different from each other at $p<0.05$. The sensitivity of the detection decreased as the concentration of gelatin was increased. At 0.4 mg/ml gelatin concentration, the sensitivity was 0.5 nM of prion, which decreased to 2 nM at 2 mg/ml gelatin concentration. From the above results it is evident that the developed biosensor can be used to detect prion in gelatin solutions. Detection technique has limitations at high gelatin concentrations; however addition of SDS improves the differences among gelatin solutions. The detection limit using this technique can be as low as 0.5nM, which decreases to 2nM for 2 mg/ml gelatin concentrations.

5.5 Dilution Studies in Baby Formula

Baby formula is a very complex medium and it contains many different proteins, fat, carbohydrate and minerals. These ingredients in the baby formula work as a scattering medium for the incident light, which adversely affect the fluorescence and emission peak of decoy. To minimize the scattering from the baby formula, serial dilutions of baby formula were done and the linear range of decoy was determined in diluted formula samples.

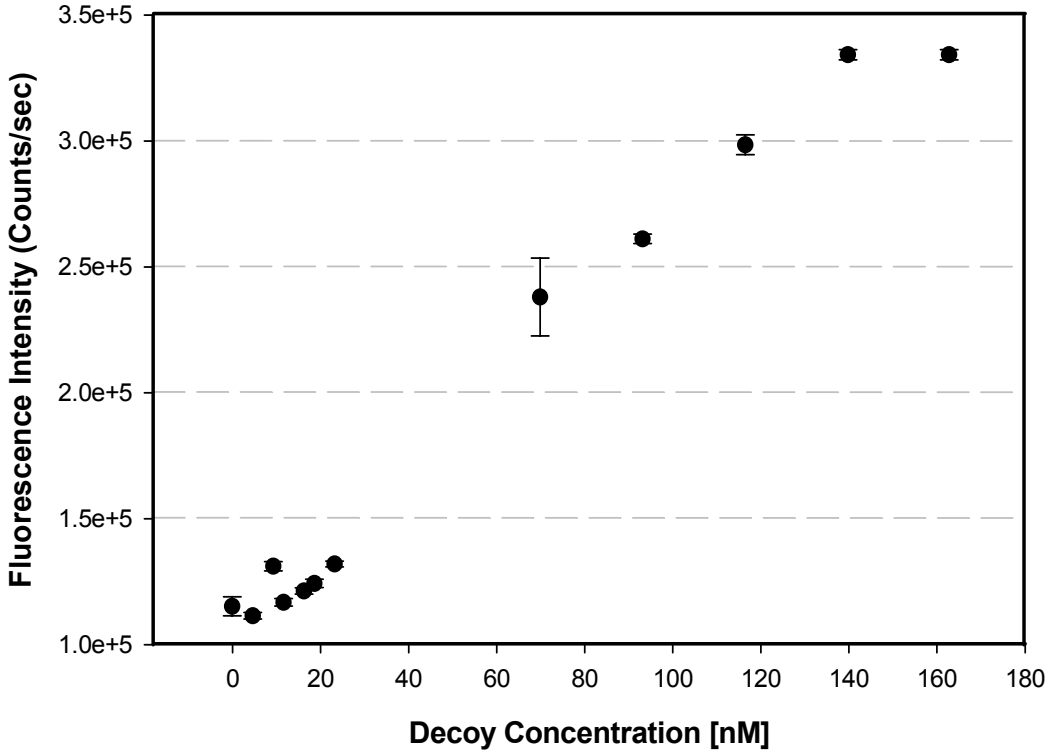

Figure 27: Linear range of decoy in full strength baby formula. Full strength baby formula is 29 gms/100ml.

Figure (27) represents the variation of fluorescence intensity with the decoy concentrations in full strength baby formula. More variation in the intensity was observed at the low concentrations of decoy. To overcome this problem, the baby formula was diluted. From the preliminary scans of baby formula it was clear that full strength formula has a fluorescence peak at 526-528 nm. Due to this inherent fluorescence peak of the baby formula, the emission peak of decoy was not visible at high concentrations and decoy fluorescence was not stable. Figure (28) represents the scans of baby formula at different concentrations. As the strength of baby formula was reduced intensity of its peak dropped and the decoy peak was more visible. Therefore it was concluded that dilution of the baby formula was necessary to conduct the experiments at low concentrations of decoy. Figure (29) represents the linear range curve of the decoy in 20 times diluted baby formula solution. At low concentration of decoy a more stable signal was observed. Variation of the intensity was linear with the concentration with R^2 as 0.994. Other linear curves were generated at different concentrations of baby formula.

5.6 Prion Detection in Baby Formula

Further experiments for prion detection were conducted in 20 times diluted baby formula. Table (7) explains the undesirable effect of baby formula on the differences between control and antibody samples of baby formula. Differences between buffer control and antibody samples were 47% as compared to 3% for the baby formula samples.

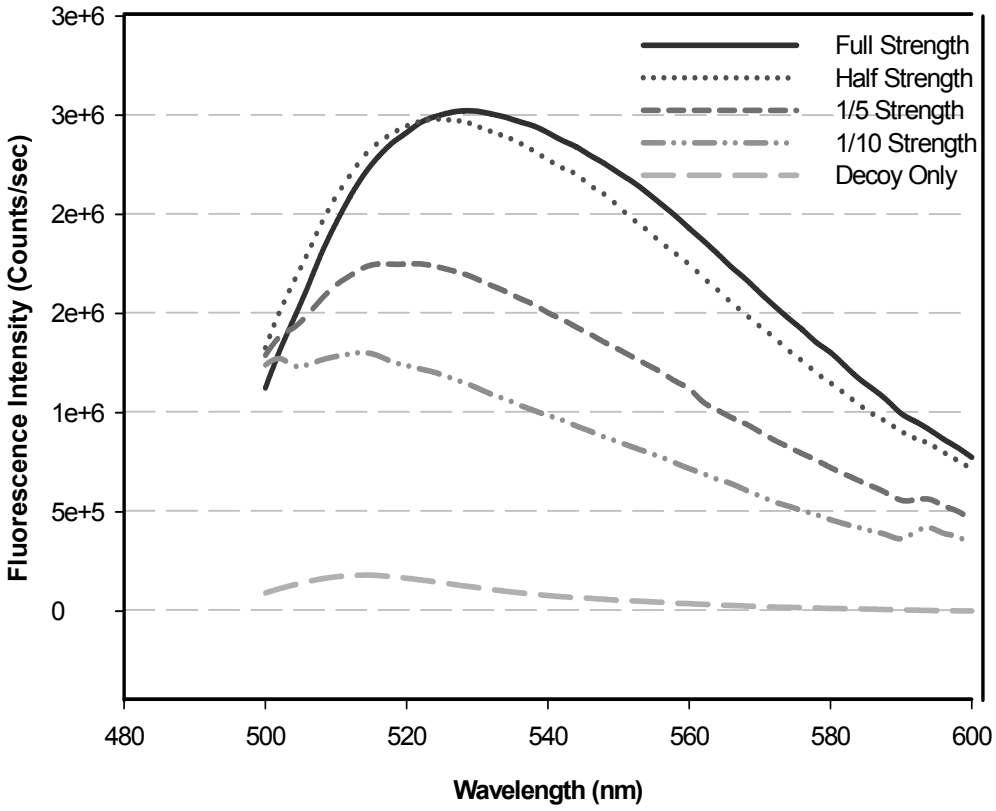

Figure 28: Influence of baby formula dilution on the decoy peak. Decoy peak was at 514 nm and baby formula has a broad peak at 526-528 nm.

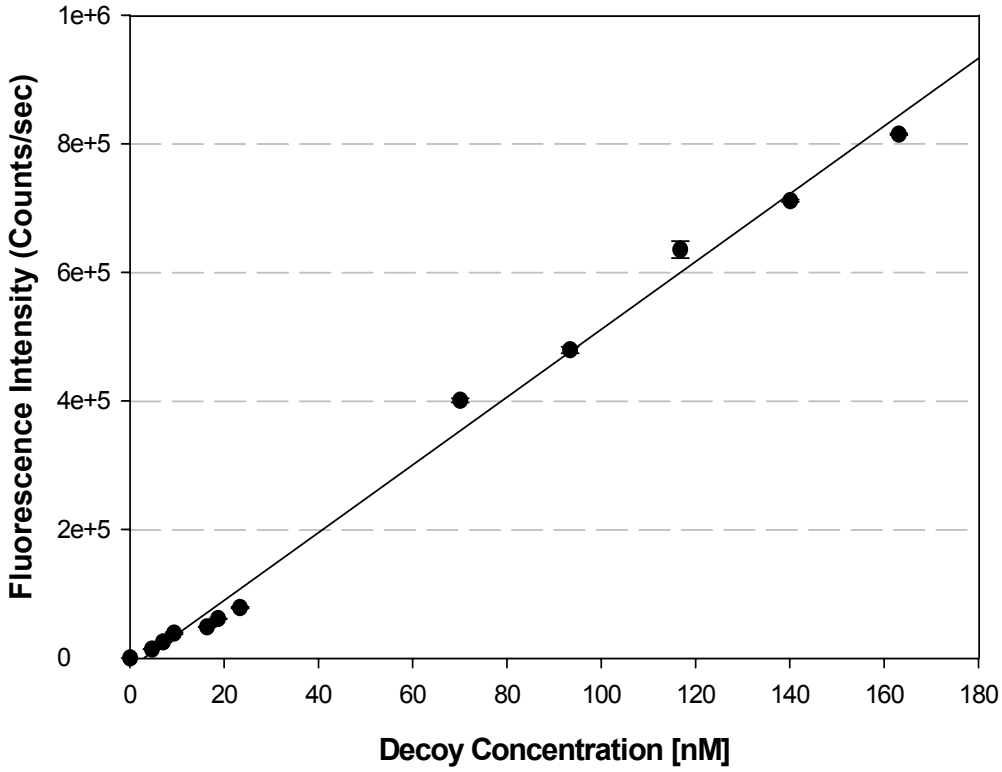

Figure 29: Decoy linear range study in 20 times diluted baby formula. R^2 value is 0.995.

Table 7:

Effect of baby formula on the percentage differences between control and antibody samples. Baby formula concentration was 1.31 mg/ml. Data Analysis (Appendix A-19).

Samples	Intensity (Counts/sec)	Differences in Intensity
Control	335180.00	-
Control	321684.00	-
Control	319093.00	-
Antibody	480693.00	43.41%
Antibody	481046.00	49.53%
Antibody	481383.00	50.85%
Baby formula control	831730.00	-
Baby formula control	845225.00	-
Baby formula control	847816.00	-
Baby formula antibody	860400.00	3.44%
Baby formula antibody	869829.00	2.91%
Baby formula antibody	878898.00	3.66%

Control = Buffer + Decoy, Antibody = Buffer + Antibody + decoy, Baby formula control = Buffer + Decoy + Baby formula, Baby formula antibody = Buffer + Decoy + Antibody + Baby formula

The 3% difference between baby formula control and antibody samples were within experimental error of the system therefore cannot be considered significantly different. A possible explanation behind this behavior of baby formula samples could be that the baby formula is binding with the decoy due to formula's sticky nature, and enough free decoy is not available for the antibody to bind. It had been cited in the literature that milk solids and proteins are used to reduce the nonspecific binding in antibody-antigen reactions (Kaur et al., 2002). The ability of milk powder to stick with the molecules serves as a good blocking agent in antibody-antigen reactions.

To reduce the possible interactions of baby formula with the decoy, a small amount (0.454 mg/ml) of Triton-X (detergent) was added to the baby formula samples. Addition of Triton-X improved the differences between the baby formula control and the baby formula antibody sample. Table (8) explains the effect of Triton-X on the baby formula samples. Percentage differences between control and antibody samples were similar for the buffer and the baby formula samples. The average percentage difference for buffer samples was 17.35 and for baby formula samples 16.12. Concentration of baby formula has an adverse effect on the intensity differences between control and antibody samples.

As the concentration of baby formula increased from 1.31 mg/ml to 9.17 mg/ml the differences between the samples decreased from 15.59% to 8.25% Figure (30 & 31). Hence, the concentrations more than 9.17 mg/ml of baby

Table 8:

Effect of Triton-X-100 on the % differences in baby formula control and antibody samples. Decoy concentration 10 nM, antibody concentration10 nM, Triton-X-100 0.454 [mg/ml] and baby formula concentration is 1.31 mg/ml. Data analysis (Appendix A-20).

Sample	Average Intensity (Counts/sec)	% Difference in intensity
Control	327150.67	-
Antibody sample	383919.00	17.35
Baby formula control	477219.67	-
Baby formula antibody	471367.67	-1.23
Baby formula Triton control	482852.67	-
Baby formula Triton antibody	560683.33	16.12

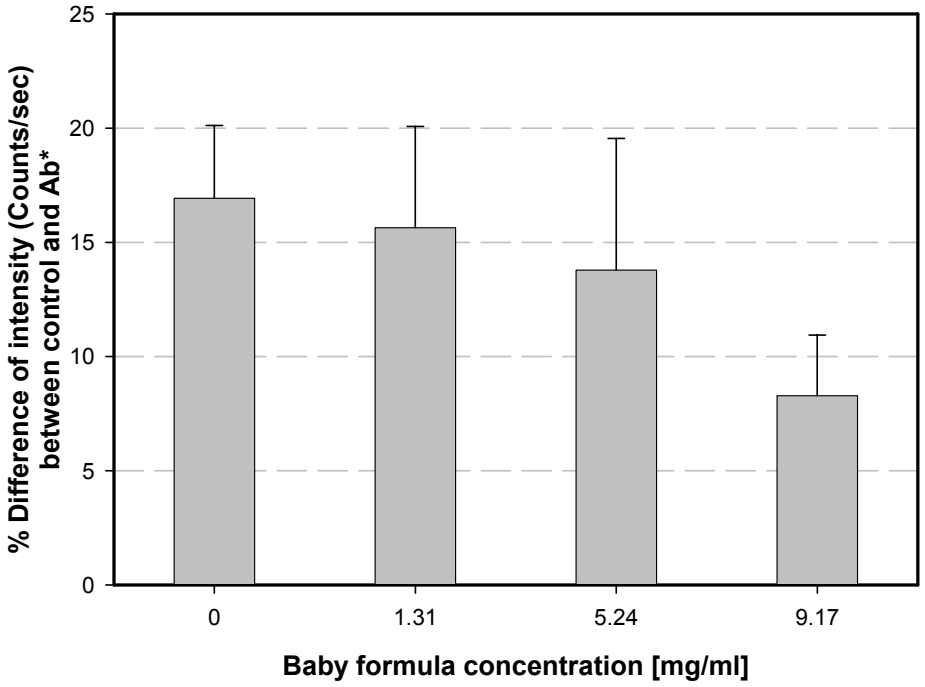

Figure 30: Effect of baby formula concentration on the % differences in the intensity of control and antibody samples. Decoy concentration 10 nM, antibody concentration 10 nM, Triton-X –100 0.454 [mg/ml]. Data analysis (Appendix A-21).

Ab*- Antibody

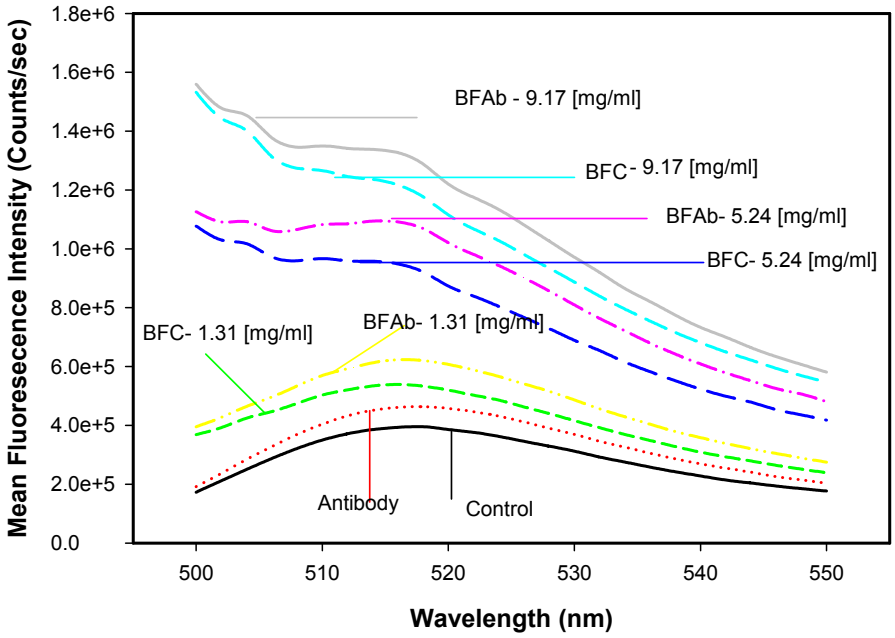

Figure 31: Scans of baby formula control and antibody samples at different concentrations.

BFC- Baby formula control, BFAb- Baby formula antibody sample.

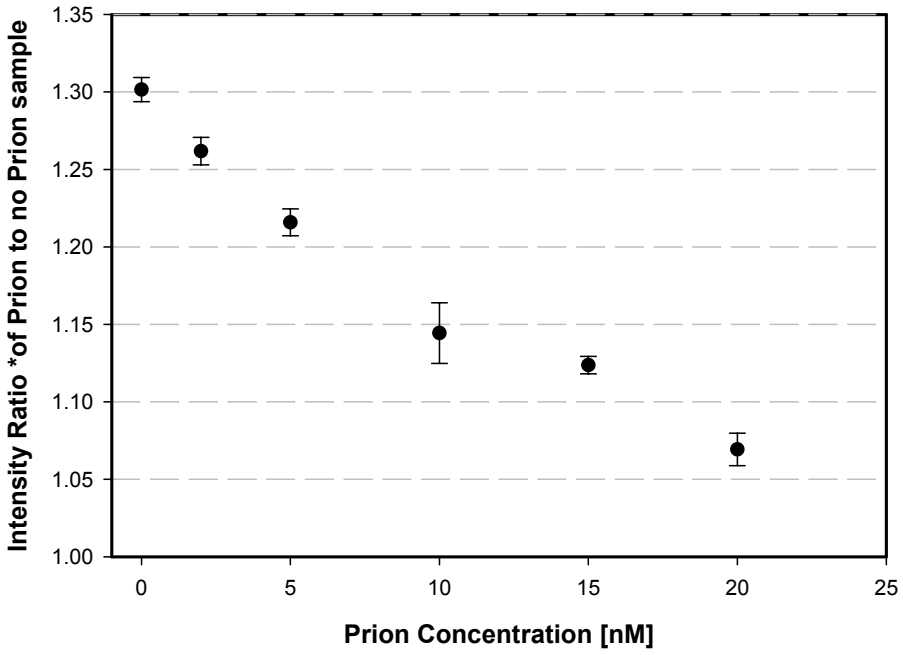

Figure 32: Prion detection curve in 1.31 mg/ml baby formula. Decoy concentration was 10 nM, antibody concentration was 10 nM. Prion was added in the increasing ratio to decoy, Triton-X was 0.454 mg/ml. Data analysis (Appendix A-22). *Intensity Ratio** is defined as the ratio of mean fluorescence intensities of prion samples to the no prion sample/antibody sample.

formula may result in very small differences between control and antibody samples, which may not be statistically significant. Figure (32) represents the prion detection curve in 1.31 mg/ml baby formula. The minimum detectable concentration of prion from no prion sample was 2 nM. All the prion concentrations were significantly different at $p<0.05$ from each other. Figure (33) represents the prion detection curve in 5.34 mg/ml baby formula solution. The lowest detectable prion concentration was 2 nM that is similar to the prion detection curve at 1.31 mg/ml baby formula concentration. All the prion concentrations except 10 and 15 nM were significantly different from each other at $p<0.05$. No difference between 10 and 15 nM prion samples can be attributed to experimental uncertainties. It can be ascertained that the developed bio-sensing technique can be used to detect prion in baby formula samples Figure (32&33). Addition of Triton-X-100 reduces non-specific interactions between the baby formula and decoy. The lowest detectable prion concentration was 2 nM.

5.7 Effect of Prion and Detergents on Decoy Fluorescence

As the concentration of prion increased, fluorescence intensity of the samples approached that of the control value. Experiments were done to establish that the change in the intensity of prion samples was due to the proposed displacement of the decoy by the prion. Table (9) shows the effect of different prion concentrations on the decoy fluorescence. The difference among the samples were not significant at $p<0.05$. Therefore, it can be said that addition of

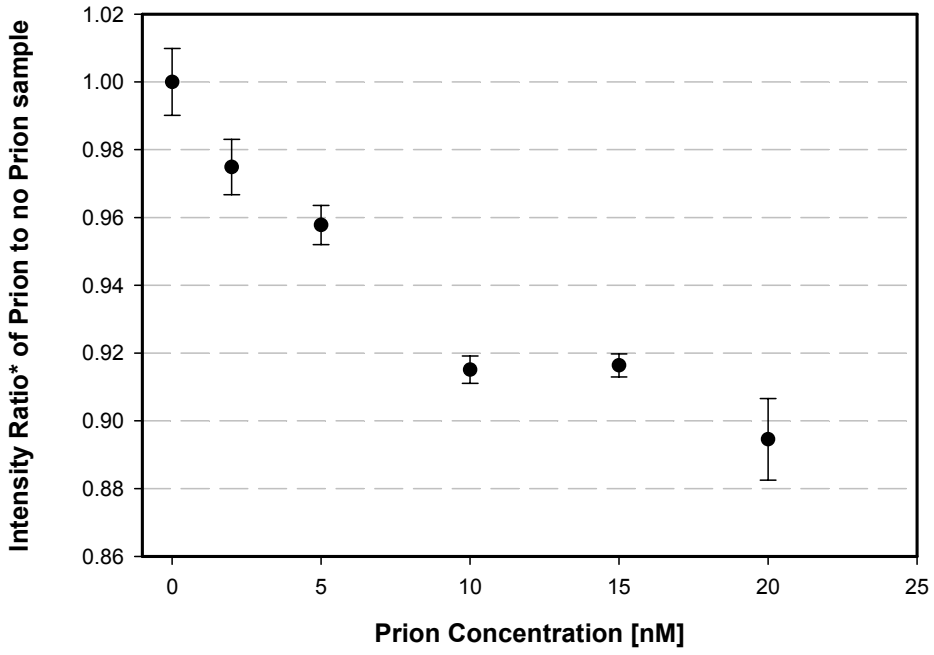

Figure 33: Prion detection curve in 5.34 mg/ml baby formula. Decoy concentration was 10 nM, antibody concentration was 10 nM. Prion was added in the increasing ratio to decoy, Triton-X was 0.454 mg/ml. Data analysis (Appendix A-23). *Intensity ratio** is defined as ratio of mean fluorescence intensities of prion samples to the no prion sample/antibody sample.

Table 9:

Effect of prion concentration on decoy fluorescence. Decoy concentration was 4 nM and experiment was done in Phosphate buffer. Data Analysis (Appendix A-24).

Prion Concentration [nM]	Average Intensity Values (counts/sec)
0	140825.00
0.5	143357.00
0.8	134954.00
1.0	143475.00
2.0	147381.00
5.0	143266.00
10.0	144110.00

prion does not change the decoy fluorescence and that change in the intensity of the prion samples was due to the displacement reaction.

Similar experiments were done to ascertain that detergents like SDS and Triton-X do not have any significant influence on the fluorescence of decoy. Results are shown in Table (10) and Figure (34 & 35); Fluorescence intensity of the decoy only in phosphate buffer was not significantly different from the solutions containing decoy and detergents like SDS and Triton-X. Figure (34) represents the scan of phosphate buffer, SDS and Triton-X. SDS and Triton-X solution were made in phosphate buffer only.

Table 10:

Effect of SDS and Triton-X on the decoy fluorescence. Decoy concentration was 10 nM. Data analysis (Appendix A-25).

Decoy Concentration [nM]	Buffer and Decoy- Intensities (Counts/sec)	Buffer SDS and decoy- Intensities (Counts/sec)	Buffer Triton and decoy - Intensities (Counts/sec)
10	359893.00	360993.00	339610.00
10	330187.00	369390.00	331484.00
10	361084.00	367008.00	349127.00

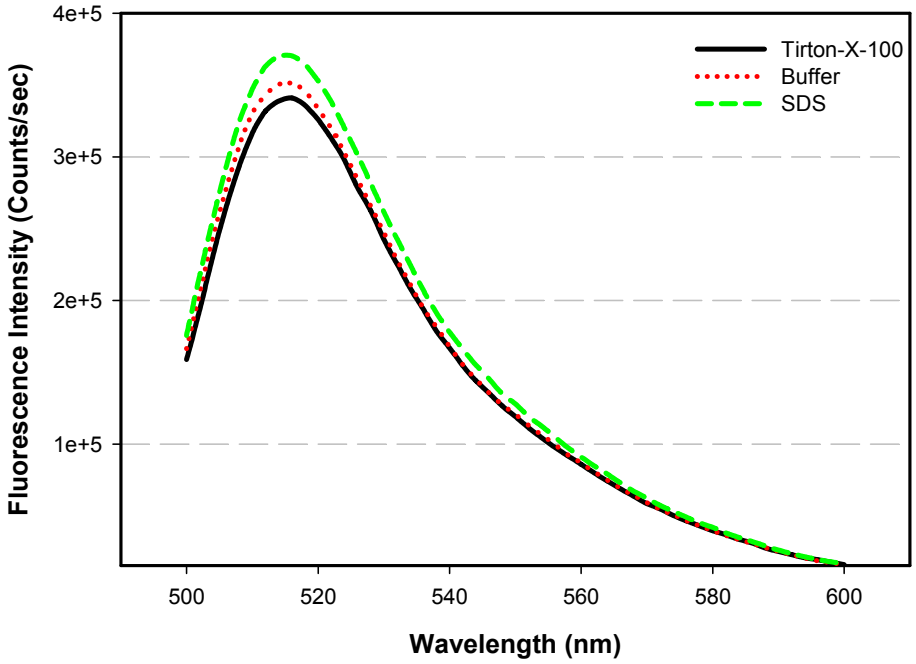

Figure 34: Effect of detergents on decoy fluorescence.

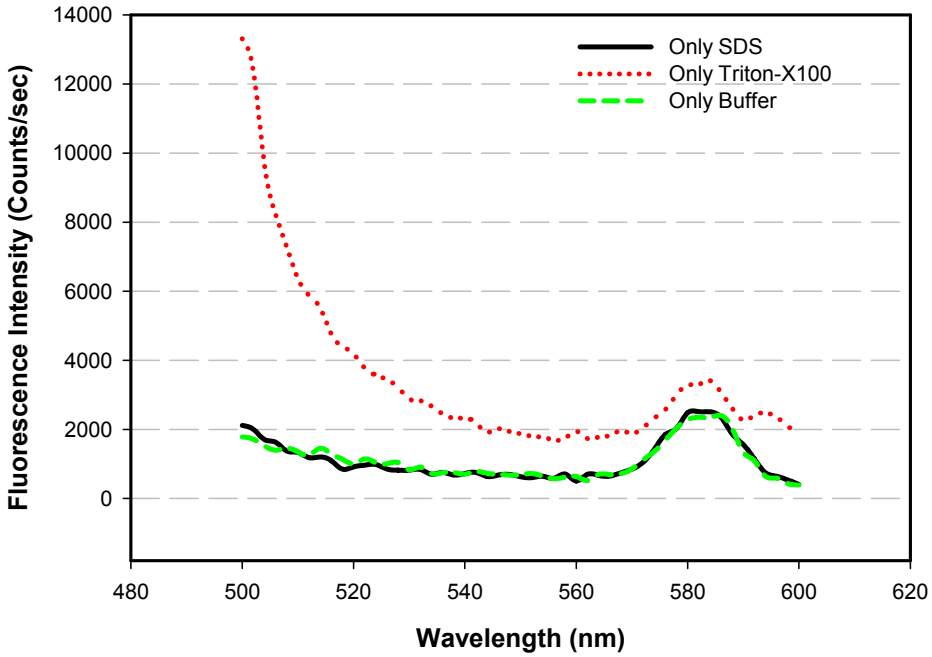

Figure 35: Scans of buffer, SDS and Triton-X-100.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of All the Results

An affinity-based biosensor was developed to detect prions (causative agent for mad cow disease). Initial experimental conditions were established in 0.1M sodium phosphate buffer. The time for the antibody decoy reaction to reach equilibrium was less than four hours. The optimum pH for the reaction was 7±0.01 and decoy was displaced by prion in less than four hours. The incubation temperature of the samples was room temperature (25±5°C) and the samples were covered in aluminum foil to prevent photo bleaching.

Once the experimental conditions for the buffer system were defined implementation of the technique was done in food systems like gelatin and baby formula. Detergents like SDS and Triton-X blocked the non-specific interactions of the gelatin and the baby formula with the decoy. Prion detection curves were generated in gelatin and baby formula. The lowest detectable concentration was 0.5 nM in gelatin and 2 nM in the case of baby formula.

The results obtained from all the experiments can be summarized as:

The decoy fluorescence was pH dependent. As the pH of the buffer was increased, the fluorescence intensity increased to a maximum value.

The fluorescence of the dye changed with the polarity of the solvent. The intensity increased in more polar solvents.

The reaction between the antibody and the decoy reached equilibrium in less than four hours. The optimum pH for the reaction was 7 because the maximum difference between the control and the antibody sample was observed at pH 7. Upon binding with the antibody, the decoy fluorescence increased (15-60%) as compared to the control sample.

High prion concentration control samples (60 nM onwards) showed an increase in the fluorescence intensity due to possible aggregation of prion in the final samples.

The prion displaced the bound decoy in less than four hours. All the prion samples incubated for four hours showed no unusual increase in the fluorescence intensity. Therefore the equilibrium time for the reaction was less than four hours.

The sensitivity of the biosensor was high for the low concentrations (less than 20 nM) of antibody and decoy. At high concentrations, saturation effect was observed. The equilibrium constant for the reaction was close to $1*10^{9}$ $mole^{-1}$

The lowest detectable prion concentration in phosphate buffer at 4 nM decoy concentration was 8 nM. In the case of 1 nM decoy concentration, it was close to 0.5 nM.

At 1 nM decoy concentration, quenching between control and antibody samples was observed. A possible explanation could be that binding of one decoy molecule to the antibody changes the affinity of the antibody for the

second decoy molecule and leads to a different binding model. It is possible that the 1nM samples need more incubation time to reach equilibrium.

Gelatin reduced the differences between control and antibody samples due to some non-specific binding.

The addition of SDS to the gelatin samples improved the differences between control and antibody samples. The minimum detectable prion concentration in gelatin samples was 0.5 nM.

The sensitivity of the detection decreased as the concentration of gelatin was increased. At 0.4 mg/ml it was 0.5 nM and 2 nM at 2 mg/ml.

Full strength baby formula has an intrinsic fluorescence peak at 526 nm. The strength of the baby formula affects the decoy fluorescence and the decoy peak decreased as the strength of the baby formula was increased.

Dilution of baby formula by twenty times produced a linear curve of decoy concentration and intensity with R^2 of 0.995. Dilutions higher than 20 times gave even better linear relationship.

The difference in the intensities of the control and antibody samples decreased in the presence of baby formula. Addition of Triton-X 100 (detergent) improved the differences between the control and antibody samples of baby formula.

The prion detection limit in 1.31 mg/ml and 5.34 mg/ml baby formula samples was between 2 to 20 nM respectively.

6.2 Specific Inferences

On the basis of the data obtained from the different experiments following inferences can be drawn:

Present detection technique can be used to detect prion in the buffer, gelatin and the baby formula.

Detection limit of the sensing technique was 8 nM in the case of buffer, 0.5 nM in the 0.4 mg/ml gelatin and 2 nM in the baby formula at 1.31 and 5.34 mg/ml concentrations.

Addition of ionic and non-ionic surfactants like SDS and Triton-X-100 facilitated the prion detection in the foods by reducing the non-specific binding.

High concentrations of the gelatin and the baby formula adversely affected the detection limit and sensing abilities of the biosensor.

6.3 Recommendations

On the basis of experimental data it can be concluded that the proposed bio-sensing technique can be used to detect prion in gelatin and baby formula samples. Detection limit of the biosensor is in the low nano molar range but still not equivalent to other detection techniques like ELISA and Western Blot.

Although the present bio-sensing scheme is the most novel method of sensing prion in foods, it requires certain modifications. How to improve the sensitivity of the bio sensor in low pico molar range, how to predict interactions of foods materials containing meat with antibody, decoy, or prion, and how to

establish a scientific hypothesis to explain odd behavior exhibited by food materials are some questions which need to be further investigated.

In the present research work, only non-infectious prion was detected using the proposed biosensor. Experiments involving infectious prion have not been done. However the present detection technique can be used to detect infectious prion using the fact that infectious prion has resistance to enzymes like Proteases. Therefore by modifying the sample preparation (pretreatment of the prion samples with Proteases) procedure biosensor can be used to differentiate between non-infectious prion from the infectious prion.

Another important aspect that has not been addressed in this research is how to extend the use of the present technique to detect BSE in live animals. A possible way would be to test the blood or cellular fluids of animals to sense the presence of prion.

In conclusion, it can be said that the proposed bio-sensing strategy can be used to detect prions in buffer as well as foods. There are certain questions like sensitivity, detection limits, extending use of technique to detect infectious prion in foods and live animals need to be addressed. The current biosensor can be used as a platform to design a more robust and sensitive sensor to detect BSE in foods and live animals.

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APPENDIX A

Statistical Analysis

TableA-1: Descriptive Statistics, ANOVA and mean comparison (LSD) for pH studies of fluorescein.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
5.00	3	6573.6800	460.46737	265.85096	5429.8156	7717.5444
6.00	3	48051.67	827.25785	477.61754	45996.6443	50106.6891
7.00	3	69380.53	762.32935	440.13106	67486.8022	71274.2644
8.00	3	97988.97	1441.11781	832.02976	94409.0316	101568.9018
9.00	3	102923.7	2175.24535	1255.878	97520.0577	108327.2757
10.00	3	54343.07	1380.59026	797.08416	50913.4903	57772.6430
Total	18	63210.26	33469.10522	7888.744	46566.4689	79854.0578

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.90E+10	5	3804538586	2239.704	.000
Within Groups	20384148	12	1698679.021		
Total	1.90E+10	17			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) PH	(J) PH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5.00	6.00	-41477.987*	1064.168	.000	-43796.6087	-39159.3646
	7.00	-62806.853*	1064.168	.000	-65125.4754	-60488.2313
	8.00	-91415.287*	1064.168	.000	-93733.9087	-89096.6646
	9.00	-96349.987*	1064.168	.000	-98668.6087	-94031.3646
	10.00	-47769.387*	1064.168	.000	-50088.0087	-45450.7646
6.00	5.00	41477.9867*	1064.168	.000	39159.3646	43796.6087
	7.00	-21328.867*	1064.168	.000	-23647.4887	-19010.2446
	8.00	-49937.300*	1064.168	.000	-52255.9220	-47618.6780
	9.00	-54872.000*	1064.168	.000	-57190.6220	-52553.3780
	10.00	-6291.4000*	1064.168	.000	-8610.0220	-3972.7780
7.00	5.00	62806.8533*	1064.168	.000	60488.2313	65125.4754
	6.00	21328.8667*	1064.168	.000	19010.2446	23647.4887
	8.00	-28608.433*	1064.168	.000	-30927.0554	-26289.8113
	9.00	-33543.133*	1064.168	.000	-35861.7554	-31224.5113
	10.00	15037.4667*	1064.168	.000	12718.8446	17356.0887
8.00	5.00	91415.2867*	1064.168	.000	89096.6646	93733.9087
	6.00	49937.3000*	1064.168	.000	47618.6780	52255.9220
	7.00	28608.4333*	1064.168	.000	26289.8113	30927.0554
	9.00	-4934.7000*	1064.168	.001	-7253.3220	-2616.0780
	10.00	43645.9000*	1064.168	.000	41327.2780	45964.5220
9.00	5.00	96349.9867*	1064.168	.000	94031.3646	98668.6087
	6.00	54872.0000*	1064.168	.000	52553.3780	57190.6220
	7.00	33543.1333*	1064.168	.000	31224.5113	35861.7554
	8.00	4934.7000*	1064.168	.001	2616.0780	7253.3220
	10.00	48580.6000*	1064.168	.000	46261.9780	50899.2220
10.00	5.00	47769.3867*	1064.168	.000	45450.7646	50088.0087
	6.00	6291.4000*	1064.168	.000	3972.7780	8610.0220
	7.00	-15037.467*	1064.168	.000	-17356.0887	-12718.8446
	8.00	-43645.900*	1064.168	.000	-45964.5220	-41327.2780
	9.00	-48580.600*	1064.168	.000	-50899.2220	-46261.9780

*. The mean difference is significant at the .05 level.

Table A-2: Descriptive Statistics, ANOVA and mean comparison (LSD) to see the effect of solvent on decoy fluorescence.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
1.00	3	69380.53	762.32935	440.13106	67486.8022	71274.2644
2.00	3	21710.70	251.86558	145.41466	21085.0312	22336.3688
3.00	3	72772.07	1059.17889	611.51722	70140.9204	75403.2129
Total	9	54621.10	24735.37841	8245.126	35607.8050	73634.3950

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.89E+09	2	2445589337	4153.415	.000
Within Groups	3532884	6	588814.079		
Total	4.89E+09	8			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) TRT	(J) TRT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	47669.8333*	626.53230	.000	46136.7640	49202.9026
	3.00	-3391.5333*	626.53230	.002	-4924.6026	-1858.4640
2.00	1.00	-47669.833*	626.53230	.000	-49202.9026	-46136.7640
	3.00	-51061.367*	626.53230	.000	-52594.4360	-49528.2974
3.00	1.00	3391.5333*	626.53230	.002	1858.4640	4924.6026
	2.00	51061.3667*	626.53230	.000	49528.2974	52594.4360

*. The mean difference is significant at the .05 level.

Table A-3: Descriptive Statistics, ANOVA and mean comparison (LSD) for binding studies at different pH values.

Descriptives

DIFFEREN

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
6.00	3	9.4033	.59652	.34440	7.9215	10.8852
7.00	3	41.0300	2.17580	1.25620	35.6250	46.4350
8.00	3	27.7200	1.66934	.96379	23.5731	31.8669
Total	9	26.0511	13.82324	4.60775	15.4256	36.6766

ANOVA

DIFFEREN

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1512.902	2	756.451	288.112	.000
Within Groups	15.753	6	2.626		
Total	1528.656	8			

Multiple Comparisons

Dependent Variable: DIFFEREN

LSD

(I) PH	(J) PH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
6.00	7.00	-31.6267*	1.32301	.000	-34.8640	-28.3894
	8.00	-18.3167*	1.32301	.000	-21.5540	-15.0794
7.00	6.00	31.6267*	1.32301	.000	28.3894	34.8640
	8.00	13.3100*	1.32301	.000	10.0727	16.5473
8.00	6.00	18.3167*	1.32301	.000	15.0794	21.5540
	7.00	-13.3100*	1.32301	.000	-16.5473	-10.0727

*. The mean difference is significant at the .05 level.

Table A-4: Descriptive Statistics, ANOVA and mean comparison (LSD) for equilibrium study for antibody antigen reaction.

Descriptives

VAR00003

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
.00	3	16.9733	6.29712	3.63564	1.3304	32.6162
1.00	3	29.6800	1.66529	.96146	25.5432	33.8168
2.00	3	20.0233	4.59970	2.65564	8.5970	31.4496
4.00	3	29.3200	4.10004	2.36716	19.1349	39.5051
5.00	3	17.8433	2.88084	1.66325	10.6869	24.9997
10.00	3	20.5633	7.08001	4.08765	2.9756	38.1511
20.00	3	18.8433	1.98651	1.14691	13.9086	23.7781
Total	21	21.8924	6.31834	1.37877	19.0163	24.7685

ANOVA

VAR00003

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	512.894	6	85.482	4.191	.013
Within Groups	285.533	14	20.395		
Total	798.427	20			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	1.00	-12.7067*	3.43887	.004	-20.3689	-5.0444
	2.00	-3.0500	3.43887	.396	-10.7123	4.6123
	4.00	-12.3467*	3.43887	.005	-20.0089	-4.6844
	5.00	-.8700	3.43887	.805	-8.5323	6.7923
1.00	.00	12.7067*	3.43887	.004	5.0444	20.3689
	2.00	9.6567*	3.43887	.019	1.9944	17.3189
	4.00	.3600	3.43887	.919	-7.3023	8.0223
	5.00	11.8367*	3.43887	.006	4.1744	19.4989
2.00	.00	3.0500	3.43887	.396	-4.6123	10.7123
	1.00	-9.6567*	3.43887	.019	-17.3189	-1.9944
	4.00	-9.2967*	3.43887	.022	-16.9589	-1.6344
	5.00	2.1800	3.43887	.540	-5.4823	9.8423
4.00	.00	12.3467*	3.43887	.005	4.6844	20.0089
	1.00	-.3600	3.43887	.919	-8.0223	7.3023
	2.00	9.2967*	3.43887	.022	1.6344	16.9589
	5.00	11.4767*	3.43887	.008	3.8144	19.1389
5.00	.00	.8700	3.43887	.805	-6.7923	8.5323
	1.00	-11.8367*	3.43887	.006	-19.4989	-4.1744
	2.00	-2.1800	3.43887	.540	-9.8423	5.4823
	4.00	-11.4767*	3.43887	.008	-19.1389	-3.8144

*. The mean difference is significant at the .05 level.

Table A-5: Descriptive Statistics, ANOVA and mean comparison (LSD) for BSA and antibody studies.

Descriptives

DIFF

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
1	3	.0000	.00000	.00000	.0000	.0000
2	3	56.8667	6.50125	3.75350	40.7167	73.0167
3	3	.3800	7.77699	4.49005	-18.9391	19.6991
Total	9	19.0822	28.78846	9.59615	-3.0465	41.2110

ANOVA

DIFF

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6424.706	2	3212.353	93.793	.000
Within Groups	205.496	6	34.249		
Total	6630.201	8			

Multiple Comparisons

Dependent Variable: DIFF

LSD

(I) SAMPLE	(J) SAMPLE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-56.8667*	4.77837	.000	-68.5589	-45.1744
	3	-.3800	4.77837	.939	-12.0723	11.3123
2	1	56.8667*	4.77837	.000	45.1744	68.5589
	3	56.4867*	4.77837	.000	44.7944	68.1789
3	1	.3800	4.77837	.939	-11.3123	12.0723
	2	-56.4867*	4.77837	.000	-68.1789	-44.7944

*. The mean difference is significant at the .05 level.

Table A-6: Descriptive Statistics, ANOVA and mean comparison (LSD) for the antibody affinity study.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
.00	3	57090.29	2621.71755	1513.649	50577.5792	63602.9941
1.00	3	79998.70	1316.13773	759.87247	76729.2326	83268.1674
2.00	3	91191.57	312.66623	180.51793	90414.8607	91968.2726
4.00	3	92632.30	531.03593	306.59374	91313.1336	93951.4664
5.00	3	92060.47	276.36355	159.55857	91373.9415	92746.9918
8.00	3	89517.93	1151.78891	664.98564	86656.7311	92379.1356
20.00	3	93775.27	1486.77292	858.38874	90081.9180	97468.6153
55.00	3	108602.1	6172.70673	3563.814	93268.2131	123935.9202
88.00	3	112862.7	3601.26663	2079.192	103916.6244	121808.7089
110.00	3	112353.0	3873.22243	2236.206	102731.3821	121974.6179
Total	30	93008.43	16211.39927	2959.783	86954.9894	99061.8613

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.46E+09	9	829347802.6	105.418	.000
Within Groups	1.57E+08	20	7867215.075		
Total	7.62E+09	29			

Table A-7: Descriptive Statistics, ANOVA and mean comparison (LSD) for Prion aggregation study.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
.00	3	63613.30	1900.40455	1097.199	58892.4334	68334.1666
10.00	3	56912.20	1864.17814	1076.284	52281.3248	61543.0752
20.00	3	74020.00	4091.10803	2362.002	63857.1243	84182.8757
60.00	3	81498.63	664.48386	383.63994	79847.9639	83149.3028
90.00	3	108978.7	4565.12095	2635.674	97638.2775	120319.0558
Total	15	77004.56	18886.20274	4876.397	66545.7295	87463.3905

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.90E+09	4	1225857424	135.887	.000
Within Groups	90211461	10	9021146.125		
Total	4.99E+09	14			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) PRIONCON	(J) PRIONCON	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	10.00	6701.1000*	2452.366	.021	1236.8888	12165.3112
	20.00	-10406.700*	2452.366	.002	-15870.9112	-4942.4888
	60.00	-17885.333*	2452.366	.000	-23349.5446	-12421.1221
	90.00	-45365.367*	2452.366	.000	-50829.5779	-39901.1554
10.00	.00	-6701.1000*	2452.366	.021	-12165.3112	-1236.8888
	20.00	-17107.800*	2452.366	.000	-22572.0112	-11643.5888
	60.00	-24586.433*	2452.366	.000	-30050.6446	-19122.2221
	90.00	-52066.467*	2452.366	.000	-57530.6779	-46602.2554
20.00	.00	10406.7000*	2452.366	.002	4942.4888	15870.9112
	10.00	17107.8000*	2452.366	.000	11643.5888	22572.0112
	60.00	-7478.6333*	2452.366	.012	-12942.8446	-2014.4221
	90.00	-34958.667*	2452.366	.000	-40422.8779	-29494.4554
60.00	.00	17885.3333*	2452.366	.000	12421.1221	23349.5446
	10.00	24586.4333*	2452.366	.000	19122.2221	30050.6446
	20.00	7478.6333*	2452.366	.012	2014.4221	12942.8446
	90.00	-27480.033*	2452.366	.000	-32944.2446	-22015.8221
90.00	.00	45365.3667*	2452.366	.000	39901.1554	50829.5779
	10.00	52066.4667*	2452.366	.000	46602.2554	57530.6779
	20.00	34958.6667*	2452.366	.000	29494.4554	40422.8779
	60.00	27480.0333*	2452.366	.000	22015.8221	32944.2446

*. The mean difference is significant at the .05 level.

Table A-8: Descriptive Statistics, ANOVA and mean comparison (LSD) for Prion aggregation in control.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
.00	3	40132.73	321.99612	185.90455	39332.8506	40932.6160
10.00	3	43974.30	1976.59573	1141.188	39064.1640	48884.4360
20.00	3	45185.03	1022.05780	590.08535	42646.1010	47723.9657
60.00	3	49166.63	1756.37711	1014.045	44803.5507	53529.7159
90.00	3	68122.73	2191.94836	1265.522	62677.6317	73567.8349
Total	15	49316.29	10270.72506	2651.890	43628.5487	55004.0246

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.45E+09	4	362734919.9	140.109	.000
Within Groups	25889425	10	2588942.497		
Total	1.48E+09	14			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) PRPCONC	(J) PRPCONC	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	10.00	-3841.5667*	1313.759	.015	-6768.8033	-914.3301
	20.00	-5052.3000*	1313.759	.003	-7979.5366	-2125.0634
	60.00	-9033.9000*	1313.759	.000	-11961.1366	-6106.6634
	90.00	-27990.000*	1313.759	.000	-30917.2366	-25062.7634
10.00	.00	3841.5667*	1313.759	.015	914.3301	6768.8033
	20.00	-1210.7333	1313.759	.378	-4137.9699	1716.5033
	60.00	-5192.3333*	1313.759	.003	-8119.5699	-2265.0967
	90.00	-24148.433*	1313.759	.000	-27075.6699	-21221.1967
20.00	.00	5052.3000*	1313.759	.003	2125.0634	7979.5366
	10.00	1210.7333	1313.759	.378	-1716.5033	4137.9699
	60.00	-3981.6000*	1313.759	.013	-6908.8366	-1054.3634
	90.00	-22937.700*	1313.759	.000	-25864.9366	-20010.4634
60.00	.00	9033.9000*	1313.759	.000	6106.6634	11961.1366
	10.00	5192.3333*	1313.759	.003	2265.0967	8119.5699
	20.00	3981.6000*	1313.759	.013	1054.3634	6908.8366
	90.00	-18956.100*	1313.759	.000	-21883.3366	-16028.8634
90.00	.00	27990.0000*	1313.759	.000	25062.7634	30917.2366
	10.00	24148.4333*	1313.759	.000	21221.1967	27075.6699
	20.00	22937.7000*	1313.759	.000	20010.4634	25864.9366
	60.00	18956.1000*	1313.759	.000	16028.8634	21883.3366

*. The mean difference is significant at the .05 level.

Table A-9: Descriptive Statistics, ANOVA and mean comparison (LSD) for Prion kinetic study.

Descriptives

DIFFEREN

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
.00	3	-14.7800	13.26193	7.65678	-47.7245	18.1645
2.00	3	-20.3467	1.98782	1.14767	-25.2847	-15.4086
4.00	3	-34.3600	3.42969	1.98013	-42.8798	-25.8402
11.00	3	-32.2233	25.02059	14.44565	-94.3779	29.9313
Total	12	-25.4275	14.86520	4.29121	-34.8724	-15.9826

ANOVA

DIFFEREN

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	795.471	3	265.157	1.297	.340
Within Groups	1635.246	8	204.406		
Total	2430.717	11			

Multiple Comparisons

Dependent Variable: DIFFEREN

LSD

(I) TIME	(J) TIME	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	2.00	5.5667	11.67350	.646	-21.3525	32.4858
	4.00	19.5800	11.67350	.132	-7.3391	46.4991
	11.00	17.4433	11.67350	.173	-9.4758	44.3625
2.00	.00	-5.5667	11.67350	.646	-32.4858	21.3525
	4.00	14.0133	11.67350	.264	-12.9058	40.9325
	11.00	11.8767	11.67350	.339	-15.0425	38.7958
4.00	.00	-19.5800	11.67350	.132	-46.4991	7.3391
	2.00	-14.0133	11.67350	.264	-40.9325	12.9058
	11.00	-2.1367	11.67350	.859	-29.0558	24.7825
11.00	.00	-17.4433	11.67350	.173	-44.3625	9.4758
	2.00	-11.8767	11.67350	.339	-38.7958	15.0425
	4.00	2.1367	11.67350	.859	-24.7825	29.0558

Table A-10: Descriptive Statistics, ANOVA and mean comparison (LSD) for decoy displacement study in phosphate buffer at 4nM decoy.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
.00	3	61918.43	703.61677	406.23333	60170.5524	63666.3143
2.00	3	60787.40	831.05742	479.81123	58722.9389	62851.8611
4.00	3	61678.77	1156.81669	667.88843	58805.0747	64552.4586
8.00	3	56828.17	662.76922	382.64999	55181.7566	58474.5767
10.00	3	54444.47	158.67143	91.60899	54050.3050	54838.6284
20.00	3	53203.53	119.75451	69.14030	52906.0466	53501.0200
40.00	3	52414.43	675.52506	390.01457	50736.3361	54092.5306
Total	21	57325.03	3949.56284	861.86527	55527.2091	59122.8480

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.05E+08	6	50843797.92	102.891	.000
Within Groups	6918146	14	494153.254		
Total	3.12E+08	20			

Multiple Comparisons

Dependent Variable: VAR00002

LSD

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	2.00	1131.0333	577.61374	.074	-127.4789	2389.5456
	4.00	239.6667	577.61374	.686	-1018.8456	1498.1789
	8.00	5090.2667*	577.61374	.000	3831.7544	6348.7789
	10.00	7473.9667*	577.61374	.000	6215.4544	8732.4789
	20.00	8714.9000*	577.61374	.000	7456.3878	9973.4122
2.00	.00	-1131.0333	577.61374	.074	-2389.5456	127.4789
	4.00	-891.3667	577.61374	.149	-2149.8789	367.1456
	8.00	3959.2333*	577.61374	.000	2700.7211	5217.7456
	10.00	6342.9333*	577.61374	.000	5084.4211	7601.4456
	20.00	7583.8667*	577.61374	.000	6325.3544	8842.3789
4.00	.00	-239.6667	577.61374	.686	-1498.1789	1018.8456
	2.00	891.3667	577.61374	.149	-367.1456	2149.8789
	8.00	4850.6000*	577.61374	.000	3592.0878	6109.1122
	10.00	7234.3000*	577.61374	.000	5975.7878	8492.8122
	20.00	8475.2333*	577.61374	.000	7216.7211	9733.7456
8.00	.00	-5090.2667*	577.61374	.000	-6348.7789	-3831.7544
	2.00	-3959.2333*	577.61374	.000	-5217.7456	-2700.7211
	4.00	-4850.6000*	577.61374	.000	-6109.1122	-3592.0878
	10.00	2383.7000*	577.61374	.001	1125.1878	3642.2122
	20.00	3624.6333*	577.61374	.000	2366.1211	4883.1456
10.00	.00	-7473.9667*	577.61374	.000	-8732.4789	-6215.4544
	2.00	-6342.9333*	577.61374	.000	-7601.4456	-5084.4211
	4.00	-7234.3000*	577.61374	.000	-8492.8122	-5975.7878
	8.00	-2383.7000*	577.61374	.001	-3642.2122	-1125.1878
	20.00	1240.9333	577.61374	.053	-17.5789	2499.4456
20.00	.00	-8714.9000*	577.61374	.000	-9973.4122	-7456.3878
	2.00	-7583.8667*	577.61374	.000	-8842.3789	-6325.3544
	4.00	-8475.2333*	577.61374	.000	-9733.7456	-7216.7211
	8.00	-3624.6333*	577.61374	.000	-4883.1456	-2366.1211
	10.00	-1240.9333	577.61374	.053	-2499.4456	17.5789

*. The mean difference is significant at the .05 level.

Table A-11: Descriptive Statistics, ANOVA and mean comparison (LSD) for decoy displacement study in phosphate buffer at 1nM decoy.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
.00	3	31216.37	933.38297	538.88891	28897.7148	33535.0185
.20	3	23049.57	587.19234	339.01566	21590.9000	24508.2333
.50	3	28219.87	530.30604	306.17233	26902.5134	29537.2199
1.00	3	29719.40	1381.86269	797.81880	26286.6628	33152.1372
2.00	3	34708.27	402.27107	232.25131	33708.9699	35707.5634
5.00	3	31926.73	211.87006	122.32324	31400.4189	32453.0477
8.00	3	47069.70	291.85097	168.50024	46344.7020	47794.6980
10.00	3	50160.63	1108.77378	640.15084	47406.2866	52914.9801
Total	24	34508.82	8973.81326	1831.772	30719.5077	38298.1257

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.84E+09	7	263188342.0	427.251	.000
Within Groups	9856070	16	616004.353		
Total	1.85E+09	23			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.20	8166.8000*	689.79124	.000	6629.8493	9703.7507
	.50	2996.5000*	689.79124	.001	1459.5493	4533.4507
	1.00	1496.9667	689.79124	.055	-39.9840	3033.9173
	2.00	-3491.9000*	689.79124	.000	-5028.8507	-1954.9493
.20	.00	-8166.8000*	689.79124	.000	-9703.7507	-6629.8493
	.50	-5170.3000*	689.79124	.000	-6707.2507	-3633.3493
	1.00	-6669.8333*	689.79124	.000	-8206.7840	-5132.8827
	2.00	-11658.700*	689.79124	.000	-13195.6507	-10121.7493
.50	.00	-2996.5000*	689.79124	.001	-4533.4507	-1459.5493
	.20	5170.3000*	689.79124	.000	3633.3493	6707.2507
	1.00	-1499.5333	689.79124	.055	-3036.4840	37.4173
	2.00	-6488.4000*	689.79124	.000	-8025.3507	-4951.4493
1.00	.00	-1496.9667	689.79124	.055	-3033.9173	39.9840
	.20	6669.8333*	689.79124	.000	5132.8827	8206.7840
	.50	1499.5333	689.79124	.055	-37.4173	3036.4840
	2.00	-4988.8667*	689.79124	.000	-6525.8173	-3451.9160
2.00	.00	3491.9000*	689.79124	.000	1954.9493	5028.8507
	.20	11658.7000*	689.79124	.000	10121.7493	13195.6507
	.50	6488.4000*	689.79124	.000	4951.4493	8025.3507
	1.00	4988.8667*	689.79124	.000	3451.9160	6525.8173

*. The mean difference is significant at the .05 level.

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) VAR00003	(J) VAR00003	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5.00	8.00	-15142.967*	549.63527	.000	-16487.8757	-13798.0576
	10.00	-18233.900*	549.63527	.000	-19578.8091	-16888.9909
8.00	5.00	15142.9667*	549.63527	.000	13798.0576	16487.8757
	10.00	-3090.9333*	549.63527	.001	-4435.8424	-1746.0243
10.00	5.00	18233.9000*	549.63527	.000	16888.9909	19578.8091
	8.00	3090.9333*	549.63527	.001	1746.0243	4435.8424

*. The mean difference is significant at the .05 level.

Table A-12: Descriptive Statistics, ANOVA and mean comparison (LSD) for decoy displacement study in 0.01mg/ml gelatin at 4nM decoy.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
.00	3	47311.20	697.25280	402.55909	45579.1280	49043.2720
2.00	3	44868.90	2865.57834	1654.442	37750.4088	51987.3912
4.00	3	43086.17	1245.39746	719.03056	39992.4279	46179.9055
8.00	3	44268.33	1059.51710	611.71249	41636.3469	46900.3197
10.00	3	40872.40	808.26129	466.64987	38864.5677	42880.2323
Total	15	44081.40	2551.94314	658.90889	42668.1810	45494.6190

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	67124636	4	16781159.06	6.978	.006
Within Groups	24049157	10	2404915.669		
Total	91173793	14			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) CONC	(J) CONC	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	2.00	2442.3000	1266.206	.083	-378.9823	5263.5823
	4.00	4225.0333*	1266.206	.008	1403.7510	7046.3157
	8.00	3042.8667*	1266.206	.037	221.5843	5864.1490
	10.00	6438.8000*	1266.206	.000	3617.5177	9260.0823
2.00	.00	-2442.3000	1266.206	.083	-5263.5823	378.9823
	4.00	1782.7333	1266.206	.189	-1038.5490	4604.0157
	8.00	600.5667	1266.206	.645	-2220.7157	3421.8490
	10.00	3996.5000*	1266.206	.010	1175.2177	6817.7823
4.00	.00	-4225.0333*	1266.206	.008	-7046.3157	-1403.7510
	2.00	-1782.7333	1266.206	.189	-4604.0157	1038.5490
	8.00	-1182.1667	1266.206	.372	-4003.4490	1639.1157
	10.00	2213.7667	1266.206	.111	-607.5157	5035.0490
8.00	.00	-3042.8667*	1266.206	.037	-5864.1490	-221.5843
	2.00	-600.5667	1266.206	.645	-3421.8490	2220.7157
	4.00	1182.1667	1266.206	.372	-1639.1157	4003.4490
	10.00	3395.9333*	1266.206	.023	574.6510	6217.2157
10.00	.00	-6438.8000*	1266.206	.000	-9260.0823	-3617.5177
	2.00	-3996.5000*	1266.206	.010	-6817.7823	-1175.2177
	4.00	-2213.7667	1266.206	.111	-5035.0490	607.5157
	8.00	-3395.9333*	1266.206	.023	-6217.2157	-574.6510

*. The mean difference is significant at the .05 level.

Table A-13: Descriptive Statistics, ANOVA and mean comparison (LSD) for decoy displacement study in 0.4mg/ml gelatin at 4nM decoy.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
0	3	186319.7	5199.93811	3002.186	173402.3043	199237.0290
2	3	168287.3	3944.69445	2277.470	158488.1691	178086.4976
4	3	162936.7	174.50597	100.75107	162503.1698	163370.1635
8	3	160861.7	3544.86704	2046.630	152055.7288	169667.6046
10	3	161429.7	3944.74110	2277.497	151630.3865	171228.9468
20	3	164868.0	466.90577	269.56817	163708.1418	166027.8582
Total	18	167450.5	9493.99088	2237.755	162729.2494	172171.7506

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.39E+09	5	278071738.2	23.507	.000
Within Groups	1.42E+08	12	11829248.11		
Total	1.53E+09	17			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) PRION	(J) PRION	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	2	18032.3333*	2808.232	.000	11913.7220	24150.9446
	4	23383.0000*	2808.232	.000	17264.3887	29501.6113
	8	25458.0000*	2808.232	.000	19339.3887	31576.6113
	10	24890.0000*	2808.232	.000	18771.3887	31008.6113
	20	21451.6667*	2808.232	.000	15333.0554	27570.2780
2	0	-18032.333*	2808.232	.000	-24150.9446	-11913.7220
	4	5350.6667	2808.232	.081	-767.9446	11469.2780
	8	7425.6667*	2808.232	.021	1307.0554	13544.2780
	10	6857.6667*	2808.232	.031	739.0554	12976.2780
	20	3419.3333	2808.232	.247	-2699.2780	9537.9446
4	0	-23383.000*	2808.232	.000	-29501.6113	-17264.3887
	2	-5350.6667	2808.232	.081	-11469.2780	767.9446
	8	2075.0000	2808.232	.474	-4043.6113	8193.6113
	10	1507.0000	2808.232	.601	-4611.6113	7625.6113
	20	-1931.3333	2808.232	.505	-8049.9446	4187.2780
8	0	-25458.000*	2808.232	.000	-31576.6113	-19339.3887
	2	-7425.6667*	2808.232	.021	-13544.2780	-1307.0554
	4	-2075.0000	2808.232	.474	-8193.6113	4043.6113
	10	-568.0000	2808.232	.843	-6686.6113	5550.6113
	20	-4006.3333	2808.232	.179	-10124.9446	2112.2780
10	0	-24890.000*	2808.232	.000	-31008.6113	-18771.3887
	2	-6857.6667*	2808.232	.031	-12976.2780	-739.0554
	4	-1507.0000	2808.232	.601	-7625.6113	4611.6113
	8	568.0000	2808.232	.843	-5550.6113	6686.6113
	20	-3438.3333	2808.232	.244	-9556.9446	2680.2780
20	0	-21451.667*	2808.232	.000	-27570.2780	-15333.0554
	2	-3419.3333	2808.232	.247	-9537.9446	2699.2780
	4	1931.3333	2808.232	.505	-4187.2780	8049.9446
	8	4006.3333	2808.232	.179	-2112.2780	10124.9446
	10	3438.3333	2808.232	.244	-2680.2780	9556.9446

*. The mean difference is significant at the .05 level.

Table A-14: Descriptive Statistics, ANOVA and mean comparison (LSD) for SDS effect on gelatin samples.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
1	3	133127.7	3396.74216	1961.110	124689.6914	141565.6420
2	3	147980.7	4776.14670	2757.510	136116.0605	159845.2728
3	3	99916.47	1079.15164	623.04849	97235.7054	102597.2279
4	3	119617.3	2231.30507	1288.245	114074.4643	125160.2024
Total	12	125160.5	18678.27238	5391.953	113292.9252	137028.1414

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.76E+09	3	1252223667	123.699	.000
Within Groups	80985451	8	10123181.31		
Total	3.84E+09	11			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) SAMPLES	(J) SAMPLES	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-14853.000*	2597.843	.000	-20843.6364	-8862.3636
	3	33211.2000*	2597.843	.000	27220.5636	39201.8364
	4	13510.3333*	2597.843	.001	7519.6969	19500.9697
2	1	14853.0000*	2597.843	.000	8862.3636	20843.6364
	3	48064.2000*	2597.843	.000	42073.5636	54054.8364
	4	28363.3333*	2597.843	.000	22372.6969	34353.9697
3	1	-33211.200*	2597.843	.000	-39201.8364	-27220.5636
	2	-48064.200*	2597.843	.000	-54054.8364	-42073.5636
	4	-19700.867*	2597.843	.000	-25691.5031	-13710.2303
4	1	-13510.333*	2597.843	.001	-19500.9697	-7519.6969
	2	-28363.333*	2597.843	.000	-34353.9697	-22372.6969
	3	19700.8667*	2597.843	.000	13710.2303	25691.5031

*. The mean difference is significant at the .05 level.

Table A-15: Descriptive Statistics, ANOVA and mean comparison (LSD) for decoy displacement in 0.4mg/ml gelatin and 0.3mg/mlSDS samples.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
.00	3	253162.3	1866.38215	1077.556	248525.9830	257798.6836
.50	3	241309.7	1485.02536	857.37979	237620.6592	244998.6742
.80	3	232625.0	2209.27160	1275.524	227136.8651	238113.1349
1.00	3	221545.7	1189.06952	686.50961	218591.8542	224499.4791
2.00	3	221991.7	4793.31914	2767.424	210084.4018	233898.9315
5.00	3	215263.0	7145.11798	4125.236	197513.5430	233012.4570
Total	18	230982.9	13777.38653	3247.361	224131.5558	237834.2220

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.05E+09	5	610970862.6	42.620	.000
Within Groups	1.72E+08	12	14335344.89		
Total	3.23E+09	17			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) CONC	(J) CONC	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.50	11852.6667*	3091.423	.002	5117.0344	18588.2989
	.80	20537.3333*	3091.423	.000	13801.7011	27272.9656
	1.00	31616.6667*	3091.423	.000	24881.0344	38352.2989
	2.00	31170.6667*	3091.423	.000	24435.0344	37906.2989
	5.00	37899.3333*	3091.423	.000	31163.7011	44634.9656
.50	.00	-11852.667*	3091.423	.002	-18588.2989	-5117.0344
	.80	8684.6667*	3091.423	.016	1949.0344	15420.2989
	1.00	19764.0000*	3091.423	.000	13028.3678	26499.6322
	2.00	19318.0000*	3091.423	.000	12582.3678	26053.6322
	5.00	26046.6667*	3091.423	.000	19311.0344	32782.2989
.80	.00	-20537.333*	3091.423	.000	-27272.9656	-13801.7011
	.50	-8684.6667*	3091.423	.016	-15420.2989	-1949.0344
	1.00	11079.3333*	3091.423	.004	4343.7011	17814.9656
	2.00	10633.3333*	3091.423	.005	3897.7011	17368.9656
	5.00	17362.0000*	3091.423	.000	10626.3678	24097.6322
1.00	.00	-31616.667*	3091.423	.000	-38352.2989	-24881.0344
	.50	-19764.000*	3091.423	.000	-26499.6322	-13028.3678
	.80	-11079.333*	3091.423	.004	-17814.9656	-4343.7011
	2.00	-446.0000	3091.423	.888	-7181.6322	6289.6322
	5.00	6282.6667	3091.423	.065	-452.9656	13018.2989
2.00	.00	-31170.667*	3091.423	.000	-37906.2989	-24435.0344
	.50	-19318.000*	3091.423	.000	-26053.6322	-12582.3678
	.80	-10633.333*	3091.423	.005	-17368.9656	-3897.7011
	1.00	446.0000	3091.423	.888	-6289.6322	7181.6322
	5.00	6728.6667	3091.423	.050	-6.9656	13464.2989
5.00	.00	-37899.333*	3091.423	.000	-44634.9656	-31163.7011
	.50	-26046.667*	3091.423	.000	-32782.2989	-19311.0344
	.80	-17362.000*	3091.423	.000	-24097.6322	-10626.3678
	1.00	-6282.6667	3091.423	.065	-13018.2989	452.9656
	2.00	-6728.6667	3091.423	.050	-13464.2989	6.9656

*. The mean difference is significant at the .05 level.

Table A-16: Descriptive Statistics, ANOVA and mean comparison (LSD) for decoy displacement in 1.0mg/ml gelatin and 0.3mg/mlSDS samples.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
.00	3	129007.7	4103.81400	2369.338	118813.2276	139202.1058
.50	3	125739.0	3212.34167	1854.646	117759.1009	133718.8991
.80	3	124674.3	1890.64019	1091.562	119977.7227	129370.9439
1.00	3	117749.3	1334.65738	770.56480	114433.8606	121064.8061
2.00	3	113333.3	2214.70931	1278.663	107831.6904	118834.9763
5.00	3	106421.0	2061.65152	1190.295	101299.5737	111542.4263
Total	18	119487.4	8359.85731	1970.437	115330.1852	123644.7037

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.10E+09	5	220947887.2	31.813	.000
Within Groups	83343207	12	6945267.222		
Total	1.19E+09	17			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) CONC	(J) CONC	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.50	3268.6667	2151.785	.155	-1419.6698	7957.0032
	.80	4333.3333	2151.785	.067	-355.0032	9021.6698
	1.00	11258.3333*	2151.785	.000	6569.9968	15946.6698
	2.00	15674.3333*	2151.785	.000	10985.9968	20362.6698
	5.00	22586.6667*	2151.785	.000	17898.3302	27275.0032
.50	.00	-3268.6667	2151.785	.155	-7957.0032	1419.6698
	.80	1064.6667	2151.785	.630	-3623.6698	5753.0032
	1.00	7989.6667*	2151.785	.003	3301.3302	12678.0032
	2.00	12405.6667*	2151.785	.000	7717.3302	17094.0032
	5.00	19318.0000*	2151.785	.000	14629.6635	24006.3365
.80	.00	-4333.3333	2151.785	.067	-9021.6698	355.0032
	.50	-1064.6667	2151.785	.630	-5753.0032	3623.6698
	1.00	6925.0000*	2151.785	.007	2236.6635	11613.3365
	2.00	11341.0000*	2151.785	.000	6652.6635	16029.3365
	5.00	18253.3333*	2151.785	.000	13564.9968	22941.6698
1.00	.00	-11258.333*	2151.785	.000	-15946.6698	-6569.9968
	.50	-7989.6667*	2151.785	.003	-12678.0032	-3301.3302
	.80	-6925.0000*	2151.785	.007	-11613.3365	-2236.6635
	2.00	4416.0000	2151.785	.063	-272.3365	9104.3365
	5.00	11328.3333*	2151.785	.000	6639.9968	16016.6698
2.00	.00	-15674.333*	2151.785	.000	-20362.6698	-10985.9968
	.50	-12405.667*	2151.785	.000	-17094.0032	-7717.3302
	.80	-11341.000*	2151.785	.000	-16029.3365	-6652.6635
	1.00	-4416.0000	2151.785	.063	-9104.3365	272.3365
	5.00	6912.3333*	2151.785	.007	2223.9968	11600.6698
5.00	.00	-22586.667*	2151.785	.000	-27275.0032	-17898.3302
	.50	-19318.000*	2151.785	.000	-24006.3365	-14629.6635
	.80	-18253.333*	2151.785	.000	-22941.6698	-13564.9968
	1.00	-11328.333*	2151.785	.000	-16016.6698	-6639.9968
	2.00	-6912.3333*	2151.785	.007	-11600.6698	-2223.9968

*. The mean difference is significant at the .05 level.

Table A-17: Descriptive Statistics, ANOVA and mean comparison (LSD) for the effect of antibody concentration on the difference between samples.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
2.00	3	124537.3	2345.05807	1353.920	118711.8862	130362.7805
3.20	3	125749.7	3297.45604	1903.787	117558.3318	133941.0016
4.00	3	135077.7	2654.08823	1532.339	128484.5460	141670.7873
Total	9	128454.9	5549.92521	1849.975	124188.8387	132720.9391

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.00E+08	2	99789981.44	12.784	.007
Within Groups	46833396	6	7805566.000		
Total	2.46E+08	8			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) ANTIBODY	(J) ANTIBODY	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
2.00	3.20	-1212.3333	2281.164	.614	-6794.1413	4369.4747
	4.00	-10540.333*	2281.164	.004	-16122.1413	-4958.5253
3.20	2.00	1212.3333	2281.164	.614	-4369.4747	6794.1413
	4.00	-9328.0000*	2281.164	.006	-14909.8080	-3746.1920
4.00	2.00	10540.3333*	2281.164	.004	4958.5253	16122.1413
	3.20	9328.0000*	2281.164	.006	3746.1920	14909.8080

*. The mean difference is significant at the .05 level.

Table A-18: Descriptive Statistics, ANOVA and mean comparison (LSD) for decoy displacement in 2.0mg/ml gelatin and 0.3mg/mlSDS samples.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
.00	3	179525.7	3792.04501	2189.338	170105.7047	188945.6287
.50	3	179253.0	2940.01701	1697.420	171949.5929	186556.4071
.80	3	181544.0	2584.19833	1491.988	175124.4955	187963.5045
1.00	3	179146.0	2900.40083	1674.547	171941.0049	186350.9951
2.00	3	168460.3	3981.73333	2298.855	158569.1594	178351.5073
5.00	3	162681.3	2105.34423	1215.521	157451.3683	167911.2983
Total	18	175101.7	7653.96339	1804.056	171295.4958	178907.9487

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.79E+08	5	5	18.064	.000
Within Groups	1.17E+08	12	12		
Total	9.96E+08	17	17		

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) CONC	(J) CONC	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.50	272.6667	2547.338	.917	-5277.5064	5822.8398
	.80	-2018.3333	2547.338	.444	-7568.5064	3531.8398
	1.00	379.6667	2547.338	.884	-5170.5064	5929.8398
	2.00	11065.3333*	2547.338	.001	5515.1602	16615.5064
	5.00	16844.3333*	2547.338	.000	11294.1602	22394.5064
.50	.00	-272.6667	2547.338	.917	-5822.8398	5277.5064
	.80	-2291.0000	2547.338	.386	-7841.1731	3259.1731
	1.00	107.0000	2547.338	.967	-5443.1731	5657.1731
	2.00	10792.6667*	2547.338	.001	5242.4936	16342.8398
	5.00	16571.6667*	2547.338	.000	11021.4936	22121.8398
.80	.00	2018.3333	2547.338	.444	-3531.8398	7568.5064
	.50	2291.0000	2547.338	.386	-3259.1731	7841.1731
	1.00	2398.0000	2547.338	.365	-3152.1731	7948.1731
	2.00	13083.6667*	2547.338	.000	7533.4936	18633.8398
	5.00	18862.6667*	2547.338	.000	13312.4936	24412.8398
1.00	.00	-379.6667	2547.338	.884	-5929.8398	5170.5064
	.50	-107.0000	2547.338	.967	-5657.1731	5443.1731
	.80	-2398.0000	2547.338	.365	-7948.1731	3152.1731
	2.00	10685.6667*	2547.338	.001	5135.4936	16235.8398
	5.00	16464.6667*	2547.338	.000	10914.4936	22014.8398
2.00	.00	-11065.333*	2547.338	.001	-16615.5064	-5515.1602
	.50	-10792.667*	2547.338	.001	-16342.8398	-5242.4936
	.80	-13083.667*	2547.338	.000	-18633.8398	-7533.4936
	1.00	-10685.667*	2547.338	.001	-16235.8398	-5135.4936
	5.00	5779.0000*	2547.338	.043	228.8269	11329.1731
5.00	.00	-16844.333*	2547.338	.000	-22394.5064	-11294.1602
	.50	-16571.667*	2547.338	.000	-22121.8398	-11021.4936
	.80	-18862.667*	2547.338	.000	-24412.8398	-13312.4936
	1.00	-16464.667*	2547.338	.000	-22014.8398	-10914.4936
	2.00	-5779.0000*	2547.338	.043	-11329.1731	-228.8269

*. The mean difference is significant at the .05 level.

Table A-19: Descriptive Statistics, ANOVA and mean comparison (LSD) for baby formula control and antibody samples.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
1.00	3	325319.0	8637.58132	4986.910	303862.0585	346775.9415
2.00	3	481040.7	345.03092	199.20369	480183.5624	481897.7710
3.00	3	841590.3	8637.01050	4986.580	820134.8098	863045.8568
4.00	3	869709.0	9249.58383	5340.250	846731.7600	892686.2400
Total	12	629414.8	243500.31823	70292.49	474702.0289	784127.4711

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.52E+11	3	2.172E+11	3699.749	.000
Within Groups	4.70E+08	8	58719902.17		
Total	6.52E+11	11			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) SAMPLES	(J) SAMPLES	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Antibody	BF Antib	-388668.33*	6256.724	.000	-403096.3659	-374240.3008
	BF Contr	-360549.67*	6256.724	.000	-374977.6992	-346121.6341
	Control	155721.67*	6256.724	.000	141293.6341	170149.6992
BF Antib	Antibody	388668.33*	6256.724	.000	374240.3008	403096.3659
	BF Contr	28118.6667*	6256.724	.002	13690.6341	42546.6992
	Control	544390.00*	6256.724	.000	529961.9674	558818.0326
BF Contr	Antibody	360549.67*	6256.724	.000	346121.6341	374977.6992
	BF Antib	-28118.667*	6256.724	.002	-42546.6992	-13690.6341
	Control	516271.33*	6256.724	.000	501843.3008	530699.3659
Control	Antibody	-155721.67*	6256.724	.000	-170149.6992	-141293.6341
	BF Antib	-544390.00*	6256.724	.000	-558818.0326	-529961.9674
	BF Contr	-516271.33*	6256.724	.000	-530699.3659	-501843.3008

Based on observed means.

*. The mean difference is significant at the .05 level.

Table A-20: Descriptive Statistics, ANOVA and mean comparison (LSD) for the effect of Triton-X-100 on baby formula control and antibody samples.

Descriptives

INTENSIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
1.00	3	327150.7	11297.58577	6522.664	299085.9078	355215.4255
2.00	3	383919.0	12462.61317	7195.293	352960.1526	414877.8474
3.00	3	477219.7	5632.56889	3251.965	463227.5899	491211.7435
4.00	3	471367.7	21305.31869	12300.63	418442.3211	524293.0123
5.00	3	482852.7	14766.05896	8525.188	446171.7427	519533.5906
6.00	3	560683.3	16849.14364	9727.858	518827.7402	602538.9265
Total	18	450532.2	78401.18543	18479.34	411544.1744	489520.1589

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.02E+11	5	2.039E+10	96.294	.000
Within Groups	2.54E+09	12	211754124.4		
Total	1.04E+11	17			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) SAMPLES	(J) SAMPLES	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Antibody	BF Ab	-87448.667*	11881.47	.000	-113336.1739	-61561.1594
	BF Cont	-93300.667*	11881.47	.000	-119188.1739	-67413.1594
	BF TRT C	-98933.667*	11881.47	.000	-124821.1739	-73046.1594
	BFTRTAb	-176764.33*	11881.47	.000	-202651.8406	-150876.8261
	Control	56768.3333*	11881.47	.000	30880.8261	82655.8406
BF Ab	Antibody	87448.6667*	11881.47	.000	61561.1594	113336.1739
	BF Cont	-5852.0000	11881.47	.631	-31739.5072	20035.5072
	BF TRT C	-11485.000	11881.47	.353	-37372.5072	14402.5072
	BFTRTAb	-89315.667*	11881.47	.000	-115203.1739	-63428.1594
	Control	144217.00*	11881.47	.000	118329.4928	170104.5072
BF Cont	Antibody	93300.6667*	11881.47	.000	67413.1594	119188.1739
	BF Ab	5852.0000	11881.47	.631	-20035.5072	31739.5072
	BF TRT C	-5633.0000	11881.47	.644	-31520.5072	20254.5072
	BFTRTAb	-83463.667*	11881.47	.000	-109351.1739	-57576.1594
	Control	150069.00*	11881.47	.000	124181.4928	175956.5072
BF TRT C	Antibody	98933.6667*	11881.47	.000	73046.1594	124821.1739
	BF Ab	11485.0000	11881.47	.353	-14402.5072	37372.5072
	BF Cont	5633.0000	11881.47	.644	-20254.5072	31520.5072
	BFTRTAb	-77830.667*	11881.47	.000	-103718.1739	-51943.1594
	Control	155702.00*	11881.47	.000	129814.4928	181589.5072
BFTRTAb	Antibody	176764.33*	11881.47	.000	150876.8261	202651.8406
	BF Ab	89315.6667*	11881.47	.000	63428.1594	115203.1739
	BF Cont	83463.6667*	11881.47	.000	57576.1594	109351.1739
	BF TRT C	77830.6667*	11881.47	.000	51943.1594	103718.1739
	Control	233532.67*	11881.47	.000	207645.1594	259420.1739
Control	Antibody	-56768.333*	11881.47	.000	-82655.8406	-30880.8261
	BF Ab	-144217.00*	11881.47	.000	-170104.5072	-118329.4928
	BF Cont	-150069.00*	11881.47	.000	-175956.5072	-124181.4928
	BF TRT C	-155702.00*	11881.47	.000	-181589.5072	-129814.4928
	BFTRTAb	-233532.67*	11881.47	.000	-259420.1739	-207645.1594

Based on observed means.

*. The mean difference is significant at the .05 level.

Table A-21: Descriptive Statistics, ANOVA and mean comparison (LSD) for the effect of baby formula concentration on control and antibody samples.

Descriptives

						95% Confidence Interval for Mean	
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound
DIFFEREN	.00	3	16.9267	3.18550	1.83915	9.0134	24.8399
	1.31	3	15.6400	4.42553	2.55508	4.6464	26.6336
	5.24	3	13.7833	5.76125	3.32626	-.5284	28.0951
	9.17	3	8.2800	2.65667	1.53383	1.6805	14.8795
	Total	12	13.6575	4.96001	1.43183	10.5061	16.8089
INTENSIT	.00	3	459698.3	11763.06212	6791.407	430477.2671	488919.3995
	1.31	3	623304.3	18207.19046	10511.93	578075.1649	668533.5018
	5.24	3	1090000	60827.62530	35118.85	938895.8021	1241104.198
	9.17	3	1336667	15275.25232	8819.171	1298720.836	1374612.497
	Total	12	877417.3	368571.95790	106397.6	643237.8837	1111596.783

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
DIFFEREN	Between Groups	130.653	3	43.551	2.489	.135
	Within Groups	139.965	8	17.496		
	Total	270.619	11			
INTENSIT	Between Groups	1.49E+12	3	4.952E+11	449.821	.000
	Within Groups	8.81E+09	8	1100801187		
	Total	1.49E+12	11			

Multiple Comparisons

LSD

Dependent Variable	(I) CONC	(J) CONC	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
DIFFEREN	.00	1.31	1.2867	3.41523	.716	-6.5889	9.1622
		5.24	3.1433	3.41523	.384	-4.7322	11.0189
		9.17	8.6467*	3.41523	.035	.7711	16.5222
	1.31	.00	-1.2867	3.41523	.716	-9.1622	6.5889
		5.24	1.8567	3.41523	.602	-6.0189	9.7322
		9.17	7.3600	3.41523	.063	-.5155	15.2355
	5.24	.00	-3.1433	3.41523	.384	-11.0189	4.7322
		1.31	-1.8567	3.41523	.602	-9.7322	6.0189
		9.17	5.5033	3.41523	.146	-2.3722	13.3789
	9.17	.00	-8.6467*	3.41523	.035	-16.5222	-.7711
		1.31	-7.3600	3.41523	.063	-15.2355	.5155
		5.24	-5.5033	3.41523	.146	-13.3789	2.3722
INTENSIT	.00	1.31	-163606.00*	27089.99	.000	-226075.6247	-101136.3753
		5.24	-630301.67*	27089.99	.000	-692771.2914	-567832.0420
		9.17	-876968.33*	27089.99	.000	-939437.9580	-814498.7086
	1.31	.00	163606.00*	27089.99	.000	101136.3753	226075.6247
		5.24	-466695.67*	27089.99	.000	-529165.2914	-404226.0420
		9.17	-713362.33*	27089.99	.000	-775831.9580	-650892.7086
	5.24	.00	630301.67*	27089.99	.000	567832.0420	692771.2914
		1.31	466695.67*	27089.99	.000	404226.0420	529165.2914
		9.17	-246666.67*	27089.99	.000	-309136.2914	-184197.0420
	9.17	.00	876968.33*	27089.99	.000	814498.7086	939437.9580
		1.31	713362.33*	27089.99	.000	650892.7086	775831.9580
		5.24	246666.67*	27089.99	.000	184197.0420	309136.2914

*. The mean difference is significant at the .05 level.

Table A-22: Descriptive Statistics, ANOVA and mean comparison (LSD) for the Prion detection in 1.31mg/ml baby formula.

Descriptive Statistics

Dependent Variable: INTENSIT

NUMBER	NAME	Mean	Std. Deviation	N
.00	CONTROL	530037.9	14073.33107	3
	Total	530037.9	14073.33107	3
1.00	0PRP	689873.3	4130.31860	3
	Total	689873.3	4130.31860	3
2.00	2PRP	668818.3	4713.31895	3
	Total	668818.3	4713.31895	3
3.00	5PRP	652251.7	17793.19596	3
	Total	652251.7	17793.19596	3
4.00	10PRP	617960.8	17054.08008	3
	Total	617960.8	17054.08008	3
5.00	15PRP	595649.6	2970.66237	3
	Total	595649.6	2970.66237	3
6.00	20PRP	566809.6	5569.84422	3
	Total	566809.6	5569.84422	3
Total	0PRP	689873.3	4130.31860	3
	10PRP	617960.8	17054.08008	3
	15PRP	595649.6	2970.66237	3
	20PRP	566809.6	5569.84422	3
	2PRP	668818.3	4713.31895	3
	5PRP	652251.7	17793.19596	3
	CONTROL	530037.9	14073.33107	3
	Total	617343.0	55270.48675	21

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.93E+10	6	9887882012	78.243	.000
Within Groups	1.77E+09	14	126374432.0		
Total	6.11E+10	20			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) NAME	(J) NAME	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0PRP	10PRP	71912.5000*	9178.759	.000	52226.0194	91598.9806
	15PRP	94223.7500*	9178.759	.000	74537.2694	113910.2306
	20PRP	123063.75*	9178.759	.000	103377.2694	142750.2306
	2PRP	21055.0000*	9178.759	.038	1368.5194	40741.4806
	5PRP	37621.6667*	9178.759	.001	17935.1860	57308.1473
	CONTROL	159835.42*	9178.759	.000	140148.9360	179521.8973
10PRP	0PRP	-71912.500*	9178.759	.000	-91598.9806	-52226.0194
	15PRP	22311.2500*	9178.759	.029	2624.7694	41997.7306
	20PRP	51151.2500*	9178.759	.000	31464.7694	70837.7306
	2PRP	-50857.500*	9178.759	.000	-70543.9806	-31171.0194
	5PRP	-34290.833*	9178.759	.002	-53977.3140	-14604.3527
	CONTROL	87922.9167*	9178.759	.000	68236.4360	107609.3973
15PRP	0PRP	-94223.750*	9178.759	.000	-113910.2306	-74537.2694
	10PRP	-22311.250*	9178.759	.029	-41997.7306	-2624.7694
	20PRP	28840.0000*	9178.759	.007	9153.5194	48526.4806
	2PRP	-73168.750*	9178.759	.000	-92855.2306	-53482.2694
	5PRP	-56602.083*	9178.759	.000	-76288.5640	-36915.6027
	CONTROL	65611.6667*	9178.759	.000	45925.1860	85298.1473
20PRP	0PRP	-123063.75*	9178.759	.000	-142750.2306	-103377.2694
	10PRP	-51151.250*	9178.759	.000	-70837.7306	-31464.7694
	15PRP	-28840.000*	9178.759	.007	-48526.4806	-9153.5194
	2PRP	-102008.75*	9178.759	.000	-121695.2306	-82322.2694
	5PRP	-85442.083*	9178.759	.000	-105128.5640	-65755.6027
	CONTROL	36771.6667*	9178.759	.001	17085.1860	56458.1473
2PRP	0PRP	-21055.000*	9178.759	.038	-40741.4806	-1368.5194
	10PRP	50857.5000*	9178.759	.000	31171.0194	70543.9806
	15PRP	73168.7500*	9178.759	.000	53482.2694	92855.2306
	20PRP	102008.75*	9178.759	.000	82322.2694	121695.2306
	5PRP	16566.6667	9178.759	.093	-3119.8140	36253.1473
	CONTROL	138780.42*	9178.759	.000	119093.9360	158466.8973
5PRP	0PRP	-37621.667*	9178.759	.001	-57308.1473	-17935.1860
	10PRP	34290.8333*	9178.759	.002	14604.3527	53977.3140
	15PRP	56602.0833*	9178.759	.000	36915.6027	76288.5640
	20PRP	85442.0833*	9178.759	.000	65755.6027	105128.5640
	2PRP	-16566.667	9178.759	.093	-36253.1473	3119.8140
	CONTROL	122213.75*	9178.759	.000	102527.2694	141900.2306
CONTROL	0PRP	-159835.42*	9178.759	.000	-179521.8973	-140148.9360
	10PRP	-87922.917*	9178.759	.000	-107609.3973	-68236.4360
	15PRP	-65611.667*	9178.759	.000	-85298.1473	-45925.1860
	20PRP	-36771.667*	9178.759	.001	-56458.1473	-17085.1860
	2PRP	-138780.42*	9178.759	.000	-158466.8973	-119093.9360
	5PRP	-122213.75*	9178.759	.000	-141900.2306	-102527.2694

Based on observed means.

*. The mean difference is significant at the .05 level.

Table A-23:

Table A-23: Descriptive Statistics, ANOVA and mean comparison (LSD) for the Prion detection in 5.14mg/ml baby formula.

Descriptive Statistics

Dependent Variable: INTENSIT

SAMPLES	NUMBER	Mean	Std. Deviation	N
0Prp	2.00	1102600	10866.92229	3
	Total	1102600	10866.92229	3
10Prp	5.00	1009000	4413.61530	3
	Total	1009000	4413.61530	3
15Prp	6.00	1010400	3750.99987	3
	Total	1010400	3750.99987	3
20Prp	7.00	986351.3	13296.45383	3
	Total	986351.3	13296.45383	3
2Prp	3.00	1074933	9027.91966	3
	Total	1074933	9027.91966	3
5Prp	4.00	1049400	17129.79860	3
	Total	1049400	17129.79860	3
Control	1.00	951061.7	10078.25726	3
	Total	951061.7	10078.25726	3
Total	1.00	951061.7	10078.25726	3
	2.00	1102600	10866.92229	3
	3.00	1074933	9027.91966	3
	4.00	1049400	17129.79860	3
	5.00	1009000	4413.61530	3
	6.00	1010400	3750.99987	3
	7.00	986351.3	13296.45383	3
	Total	1026249	50595.32519	21

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.96E+10	6	8264643007	71.872	.000
Within Groups	1.61E+09	14	114991469.6		
Total	5.12E+10	20			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) SAMPLES	(J) SAMPLES	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0Prp	10Prp	93600.0000*	8755.626	.000	74821.0508	112378.9492
	15Prp	92200.0000*	8755.626	.000	73421.0508	110978.9492
	20Prp	116248.67*	8755.626	.000	97469.7174	135027.6159
	2Prp	27666.6667*	8755.626	.007	8887.7174	46445.6159
	5Prp	53200.0000*	8755.626	.000	34421.0508	71978.9492
	Control	151538.33*	8755.626	.000	132759.3841	170317.2826
10Prp	0Prp	-93600.000*	8755.626	.000	-112378.9492	-74821.0508
	15Prp	-1400.0000	8755.626	.875	-20178.9492	17378.9492
	20Prp	22648.6667*	8755.626	.022	3869.7174	41427.6159
	2Prp	-65933.333*	8755.626	.000	-84712.2826	-47154.3841
	5Prp	-40400.000*	8755.626	.000	-59178.9492	-21621.0508
	Control	57938.3333*	8755.626	.000	39159.3841	76717.2826
15Prp	0Prp	-92200.000*	8755.626	.000	-110978.9492	-73421.0508
	10Prp	1400.0000	8755.626	.875	-17378.9492	20178.9492
	20Prp	24048.6667*	8755.626	.016	5269.7174	42827.6159
	2Prp	-64533.333*	8755.626	.000	-83312.2826	-45754.3841
	5Prp	-39000.000*	8755.626	.001	-57778.9492	-20221.0508
	Control	59338.3333*	8755.626	.000	40559.3841	78117.2826
20Prp	0Prp	-116248.67*	8755.626	.000	-135027.6159	-97469.7174
	10Prp	-22648.667*	8755.626	.022	-41427.6159	-3869.7174
	15Prp	-24048.667*	8755.626	.016	-42827.6159	-5269.7174
	2Prp	-88582.000*	8755.626	.000	-107360.9492	-69803.0508
	5Prp	-63048.667*	8755.626	.000	-81827.6159	-44269.7174
	Control	35289.6667*	8755.626	.001	16510.7174	54068.6159
2Prp	0Prp	-27666.667*	8755.626	.007	-46445.6159	-8887.7174
	10Prp	65933.3333*	8755.626	.000	47154.3841	84712.2826
	15Prp	64533.3333*	8755.626	.000	45754.3841	83312.2826
	20Prp	88582.0000*	8755.626	.000	69803.0508	107360.9492
	5Prp	25533.3333*	8755.626	.011	6754.3841	44312.2826
	Control	123871.67*	8755.626	.000	105092.7174	142650.6159
5Prp	0Prp	-53200.000*	8755.626	.000	-71978.9492	-34421.0508
	10Prp	40400.0000*	8755.626	.000	21621.0508	59178.9492
	15Prp	39000.0000*	8755.626	.001	20221.0508	57778.9492
	20Prp	63048.6667*	8755.626	.000	44269.7174	81827.6159
	2Prp	-25533.333*	8755.626	.011	-44312.2826	-6754.3841
	Control	98338.3333*	8755.626	.000	79559.3841	117117.2826
Control	0Prp	-151538.33*	8755.626	.000	-170317.2826	-132759.3841
	10Prp	-57938.333*	8755.626	.000	-76717.2826	-39159.3841
	15Prp	-59338.333*	8755.626	.000	-78117.2826	-40559.3841
	20Prp	-35289.667*	8755.626	.001	-54068.6159	-16510.7174
	2Prp	-123871.67*	8755.626	.000	-142650.6159	-105092.7174
	5Prp	-98338.333*	8755.626	.000	-117117.2826	-79559.3841

Based on observed means.

*. The mean difference is significant at the .05 level.

Table A-24: Descriptive Statistics, ANOVA and mean comparison (LSD) for the effect of Prion concentration on decoy fluorescence.

Descriptive Statistics

Dependent Variable: DIFFEREN

NAME	NUMBER	Mean	Std. Deviation	N
0.5Prp	2.00	1.8333	2.17845	3
	Total	1.8333	2.17845	3
0.8Prp	3.00	-4.1233	2.78687	3
	Total	-4.1233	2.78687	3
1.0Prp	4.00	1.9967	6.83714	3
	Total	1.9967	6.83714	3
10.0Prp	7.00	2.4733	7.45156	3
	Total	2.4733	7.45156	3
2.0Prp	5.00	4.7333	4.50891	3
	Total	4.7333	4.50891	3
5.0Prp	6.00	1.8200	5.50564	3
	Total	1.8200	5.50564	3
noPrp	1.00	.0000	.00000	3
	Total	.0000	.00000	3
Total	1.00	.0000	.00000	3
	2.00	1.8333	2.17845	3
	3.00	-4.1233	2.78687	3
	4.00	1.9967	6.83714	3
	5.00	4.7333	4.50891	3
	6.00	1.8200	5.50564	3
	7.00	2.4733	7.45156	3
	Total	1.2476	4.83072	21

ANOVA

DIFFEREN

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	135.864	6	22.644	.958	.487
Within Groups	330.854	14	23.632		
Total	466.718	20			

Multiple Comparisons

Dependent Variable: DIFFEREN

LSD

(I) NAME	(J) NAME	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.5Prp	0.8Prp	5.9567	3.96925	.156	-2.5565	14.4699
	1.0Prp	-.1633	3.96925	.968	-8.6765	8.3499
	10.0Prp	-.6400	3.96925	.874	-9.1532	7.8732
	2.0Prp	-2.9000	3.96925	.477	-11.4132	5.6132
	5.0Prp	.0133	3.96925	.997	-8.4999	8.5265
	noPrp	1.8333	3.96925	.651	-6.6799	10.3465
0.8Prp	0.5Prp	-5.9567	3.96925	.156	-14.4699	2.5565
	1.0Prp	-6.1200	3.96925	.145	-14.6332	2.3932
	10.0Prp	-6.5967	3.96925	.119	-15.1099	1.9165
	2.0Prp	-8.8567*	3.96925	.043	-17.3699	-.3435
	5.0Prp	-5.9433	3.96925	.157	-14.4565	2.5699
	noPrp	-4.1233	3.96925	.316	-12.6365	4.3899
1.0Prp	0.5Prp	.1633	3.96925	.968	-8.3499	8.6765
	0.8Prp	6.1200	3.96925	.145	-2.3932	14.6332
	10.0Prp	-.4767	3.96925	.906	-8.9899	8.0365
	2.0Prp	-2.7367	3.96925	.502	-11.2499	5.7765
	5.0Prp	.1767	3.96925	.965	-8.3365	8.6899
	noPrp	1.9967	3.96925	.623	-6.5165	10.5099
10.0Prp	0.5Prp	.6400	3.96925	.874	-7.8732	9.1532
	0.8Prp	6.5967	3.96925	.119	-1.9165	15.1099
	1.0Prp	.4767	3.96925	.906	-8.0365	8.9899
	2.0Prp	-2.2600	3.96925	.578	-10.7732	6.2532
	5.0Prp	.6533	3.96925	.872	-7.8599	9.1665
	noPrp	2.4733	3.96925	.543	-6.0399	10.9865
2.0Prp	0.5Prp	2.9000	3.96925	.477	-5.6132	11.4132
	0.8Prp	8.8567*	3.96925	.043	.3435	17.3699
	1.0Prp	2.7367	3.96925	.502	-5.7765	11.2499
	10.0Prp	2.2600	3.96925	.578	-6.2532	10.7732
	5.0Prp	2.9133	3.96925	.475	-5.5999	11.4265
	noPrp	4.7333	3.96925	.253	-3.7799	13.2465
5.0Prp	0.5Prp	-.0133	3.96925	.997	-8.5265	8.4999
	0.8Prp	5.9433	3.96925	.157	-2.5699	14.4565
	1.0Prp	-.1767	3.96925	.965	-8.6899	8.3365
	10.0Prp	-.6533	3.96925	.872	-9.1665	7.8599
	2.0Prp	-2.9133	3.96925	.475	-11.4265	5.5999
	noPrp	1.8200	3.96925	.654	-6.6932	10.3332
noPrp	0.5Prp	-1.8333	3.96925	.651	-10.3465	6.6799
	0.8Prp	4.1233	3.96925	.316	-4.3899	12.6365
	1.0Prp	-1.9967	3.96925	.623	-10.5099	6.5165
	10.0Prp	-2.4733	3.96925	.543	-10.9865	6.0399
	2.0Prp	-4.7333	3.96925	.253	-13.2465	3.7799
	5.0Prp	-1.8200	3.96925	.654	-10.3332	6.6932

Based on observed means.

*. The mean difference is significant at the .05 level.

Table A-25: Descriptive Statistics, ANOVA and mean comparison (LSD) for the effect of detergents on decoy fluorescence.

Descriptive Statistics

Dependent Variable: INTENSIT

SAMPLE	NUMBER	Mean	Std. Deviation	N
buffer	1.00	350388.0	17504.71139	3
	Total	350388.0	17504.71139	3
SDS	2.00	365797.0	4327.50425	3
	Total	365797.0	4327.50425	3
TRITON	3.00	340073.7	8830.63431	3
	Total	340073.7	8830.63431	3
Total	1.00	350388.0	17504.71139	3
	2.00	365797.0	4327.50425	3
	3.00	340073.7	8830.63431	3
	Total	352086.2	15048.90886	9

ANOVA

INTENSIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.01E+09	2	502756315.4	3.741	.088
Within Groups	8.06E+08	6	134374105.4		
Total	1.81E+09	8			

Multiple Comparisons

Dependent Variable: INTENSIT

LSD

(I) SAMPLE	(J) SAMPLE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
buffer	SDS	-15409.000	9464.816	.155	-38568.5698	7750.5698
	TRITON	10314.3333	9464.816	.318	-12845.2365	33473.9031
SDS	buffer	15409.0000	9464.816	.155	-7750.5698	38568.5698
	TRITON	25723.3333*	9464.816	.035	2563.7635	48882.9031
TRITON	buffer	-10314.333	9464.816	.318	-33473.9031	12845.2365
	SDS	-25723.333*	9464.816	.035	-48882.9031	-2563.7635

Based on observed means.

*. The mean difference is significant at the .05 level.

Appendix B

Data Tables

pH Values	Intensity Values (counts/sec)	Coefficient of Variation
5.00	7046.45	7.00%
5.00	6548.00	
5.00	6126.59	
6.00	48785.40	1.72%
6.00	47155.10	
6.00	48214.50	
7.00	69602.70	1.10%
7.00	68531.80	
7.00	70007.10	
8.00	99592.40	1.47%
8.00	97572.70	
8.00	96801.80	
9.00	105234.00	2.11%
9.00	100915.00	
9.00	102622.00	
10.00	55917.30	2.54%
10.00	53773.60	
10.00	53338.30	

Table (B-1): pH dependence of decoy in phosphate buffer solution.

Solvent	Intensity (counts/sec)
Phosphate Buffer	69602.70
Phosphate Buffer	68531.80
Phosphate Buffer	70007.10
Ethyl Alcohol	73826.70
Ethyl Alcohol	72781.10
Ethyl Alcohol	71708.40
DMSO	21727.40
DMSO	21953.80
DMSO	21450.90

Table (B-2): Dependence of fluoresce on the polarity of the solvent.

pH Values	Samples	Intensity (counts/sec)	% Difference of intensity (counts/sec)
6.00	Control	65939.60	
6.00	Control	65243.00	
6.00	Control	64391.50	
6.00	Antibody	59565.20	-9.67%
6.00	Antibody	58836.50	-9.82%
6.00	Antibody	58778.10	-8.72%
7.00	Control	90935.40	
7.00	Control	89577.00	
7.00	Control	88244.10	
7.00	Antibody	130097.0	43.07%
7.00	Antibody	126553.0	41.28%
7.00	Antibody	122433.0	38.74%
8.00	Control	135670.0	
8.00	Control	132334.0	
8.00	Control	129929.0	
8.00	Antibody	174266.0	28.45%
8.00	Antibody	166488.0	25.81%
8.00	Antibody	167481.0	28.90%

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Table (B-3): Effect of pH on the % difference of intensity between control and antibody samples.

Time (Hours)	Control Intensity Values (Average) (counts/sec)	Antibody Intensity Values (Average) (counts/sec)	% Difference of intensity (counts/sec)
0.00	38084.30	44536.20	16.94
1.00	35938.60	47376.30	31.83
2.00	38763.40	46494.80	19.95
4.00	37158.80	48081.60	29.39
5.00	39861.30	46960.40	17.81
10.00	39488.00	47614.50	20.58
20.00	32101.87	37832.09	18.85

Table (B-4): Kinetic Study for antibody decoy reaction. Equilibrium time was less than 4 hours.

Sample	Intensity (counts/sec)	Average % Difference in intensity from control (counts/sec)
Control	53674.20	
Control	53163.50	
Control	51717.90	
Antibody Sample	80421.10	56.79
Antibody Sample	84062.40	
Antibody Sample	84120.10	
BSA Sample	50420.50	0.28
BSA Sample	52197.40	
BSA Sample	56380.60	

Table (B-5): Specificity of reaction between antibody and decoy. BSA does have the same effect.

Antibody Concentration (nM)	Average Intensity (counts/sec)	Average % Difference from 0 antibody sample (counts/sec)
0.00	57090.29	0.00
1.00	79998.70	40.13
2.00	91191.57	59.73
4.00	89298.97	56.42
5.00	89517.93	56.80
8.00	92060.47	61.25
20.00	93775.27	64.26
55.00	97514.30	70.81
88.00	109684.00	92.12
110.00	118865.00	108.21

Table (B-6): Effect of antibody concentration on decoy fluorescence. Data was analyzed to determine equilibrium constant value for the reaction between antibody and decoy.

Time(Hours)	Average Control Intensity (counts/sec)	Antibody Sample Intensity (counts/sec)	Antibody and Prion Sample Intensity (counts/sec)
0.00	72201.57	94951.57	84370.67
2.00	74123.70	101316.74	86229.83
4.00	76325.83	106597.92	80312.50
11.00	66913.60	102696.45	81007.63

Table (B-7): Prion detection kinetic study. Equilibrium time for the reaction was less than 4 hours.

Samples	Prion Concentration (nM)	Intensity Values (counts/sec)
Control	-	49736.20
Control	-	48200.80
Control	-	48805.90
Antibody	0.00	61512.20
Antibody	0.00	62730.90
Antibody	0.00	61513.20
Antibody and Prion	2.00	60273.60
Antibody and Prion	2.00	60342.40
Antibody and Prion	2.00	61746.20
Antibody and Prion	4.00	60786.20
Antibody and Prion	4.00	62985.70
Antibody and Prion	4.00	61264.40
Antibody and Prion	8.00	56693.00
Antibody and Prion	8.00	57548.10
Antibody and Prion	8.00	56243.40
Antibody and Prion	10.00	54411.30
Antibody and Prion	10.00	54617.10
Antibody and Prion	10.00	54305.00
Antibody and Prion	20.00	53136.10
Antibody and Prion	20.00	53341.80
Antibody and Prion	20.00	53132.70

Table (B-8): Prion detection data in 0.1M phosphate buffer. Decoy and antibody concentration was 4nM.

Sample	Prion Concentration (nM)	Intensity Values (counts/sec)
Control	-	36002.00
Control	-	36477.60
Control	-	41798.80
Antibody	0.00	52587.90
Antibody	0.00	52248.80
Antibody	0.00	52492.00
Antibody and Prion	2.00	49011.10
Antibody and Prion	2.00	50889.40
Antibody and Prion	2.00	48344.40
Antibody and Prion	3.00	45567.10
Antibody and Prion	3.00	46984.30
Antibody and Prion	3.00	42006.60
Antibody and Prion	4.00	46372.90
Antibody and Prion	4.00	46171.40
Antibody and Prion	4.00	39816.90
Antibody and Prion	7.00	46239.70
Antibody and Prion	7.00	44594.50
Antibody and Prion	7.00	44864.10
Antibody and Prion	8.00	39503.80
Antibody and Prion	8.00	39255.40
Antibody and Prion	8.00	40450.10

Table (B-9): Prion detection data in 0.1M phosphate buffer. Antibody and decoy concentration was 4nM.

Samples	Prion Concentration (nM)	Intensity Values (counts/sec)
Control	-	47568.50
Control	-	46670.00
Control	-	51813.80
Antibody	0.00	31448.90
Antibody	0.00	32011.50
Antibody	0.00	30188.70
Antibody and Prion	0.20	23538.80
Antibody and Prion	0.20	23211.50
Antibody and Prion	0.20	22398.40
Antibody and Prion	0.50	27609.80
Antibody and Prion	0.50	28570.60
Antibody and Prion	0.50	28479.20
Antibody and Prion	1.00	30991.40
Antibody and Prion	1.00	29917.70
Antibody and Prion	1.00	28249.10
Antibody and Prion	2.00	34623.40
Antibody and Prion	2.00	35146.20
Antibody and Prion	2.00	34355.20
Antibody and Prion	5.00	32136.90
Antibody and Prion	5.00	31930.10
Antibody and Prion	5.00	31713.20
Antibody and Prion	8.00	47008.20
Antibody and Prion	8.00	47387.40
Antibody and Prion	8.00	46813.50

Table (B-10): Prion detection data in 0.1M phosphate buffer. Decoy and antibody concentration was 1 nM.

Samples	Prion Concentration (nM)	Intensity Values (counts/sec)
Gelatin Control	-	38075.10
Gelatin Control	-	37422.40
Gelatin Control	-	37959.50
Gelatin Antibody	0.00	47800.90
Gelatin Antibody	0.00	47619.80
Gelatin Antibody	0.00	46512.90
Gelatin Antibody Prion	2.00	46485.60
Gelatin Antibody Prion	2.00	46560.80
Gelatin Antibody Prion	2.00	44560.30
Gelatin Antibody Prion	4.00	44280.60
Gelatin Antibody Prion	4.00	43182.50
Gelatin Antibody Prion	4.00	41795.40
Gelatin Antibody Prion	8.00	43356.40
Gelatin Antibody Prion	8.00	45430.60
Gelatin Antibody Prion	8.00	44018.00
Gelatin Antibody Prion	10.00	40685.00
Gelatin Antibody Prion	10.00	41757.90
Gelatin Antibody Prion	10.00	40174.30

Table (B-11): Prion detection data in 0.01mg/ml gelatin solution. Decoy and antibody concentration was 4nM.

Samples	Prion Concentration (nM)	Intensity Values (counts/sec)
Gelatin Control	-	170769.00
Gelatin Control	-	165687.00
Gelatin Control	-	167010.00
Gelatin Antibody	0.00	183336.00
Gelatin Antibody	0.00	183299.00
Gelatin Antibody	0.00	192324.00
Gelatin Antibody Prion	2.00	169690.00
Gelatin Antibody Prion	2.00	163833.00
Gelatin Antibody Prion	2.00	171339.00
Gelatin Antibody Prion	4.00	162740.00
Gelatin Antibody Prion	4.00	163073.00
Gelatin Antibody Prion	4.00	162997.00
Gelatin Antibody Prion	8.00	162758.00
Gelatin Antibody Prion	8.00	163055.00
Gelatin Antibody Prion	8.00	156772.00
Gelatin Antibody Prion	10.00	165738.00
Gelatin Antibody Prion	10.00	160556.00
Gelatin Antibody Prion	10.00	157995.00
Gelatin Antibody Prion	20.00	165364.00
Gelatin Antibody Prion	20.00	164803.00
Gelatin Antibody Prion	20.00	164437.00

Table (B-12): Prion detection data in 0.4mg/ml gelatin solution. Decoy and antibody concentration was 4nM.

Samples	Prion Concentration (nM)	Intensity Values (counts/sec)
Gelatin Control	-	170487.99
Gelatin Control	-	172644.21
Gelatin Control	-	164488.32
Gelatin Antibody	0.00	204104.61
Gelatin Antibody	0.00	204275.52
Gelatin Antibody	0.00	206804.34
Gelatin Antibody Prion	0.50	196413.66
Gelatin Antibody Prion	0.50	194109.21
Gelatin Antibody Prion	0.50	195859.62
Gelatin Antibody Prion	0.80	188546.94
Gelatin Antibody Prion	0.80	186579.45
Gelatin Antibody Prion	0.80	190152.36
Gelatin Antibody Prion	1.00	179662.86
Gelatin Antibody Prion	1.00	178400.88
Gelatin Antibody Prion	1.00	180292.23
Gelatin Antibody Prion	2.00	183837.60
Gelatin Antibody Prion	2.00	179512.20
Gelatin Antibody Prion	2.00	176089.95
Gelatin Antibody Prion	5.00	169876.44
Gelatin Antibody Prion	5.00	180895.68
Gelatin Antibody Prion	5.00	172316.97

Table (B-13): Prion detection data in 0.4mg/ml gelatin and 0.3mg/ml SDS solution. Decoy and antibody concentration was 4nM.

Samples	Prion Concentration (nM)	Intensity Values (counts/sec)
Gelatin Control	-	169402.80
Gelatin Control	-	171820.30
Gelatin Control	-	163492.40
Gelatin Antibody	0.00	224029.70
Gelatin Antibody	0.00	215841.40
Gelatin Antibody	0.00	210327.60
Gelatin Antibody Prion	0.50	217294.60
Gelatin Antibody Prion	0.50	209497.70
Gelatin Antibody Prion	0.50	206932.30
Gelatin Antibody Prion	0.80	209259.10
Gelatin Antibody Prion	0.80	206377.90
Gelatin Antibody Prion	0.80	212721.60
Gelatin Antibody Prion	1.00	198791.00
Gelatin Antibody Prion	1.00	195254.60
Gelatin Antibody Prion	1.00	199411.00
Gelatin Antibody Prion	2.00	193519.20
Gelatin Antibody Prion	2.00	186281.80
Gelatin Antibody Prion	2.00	191399.00
Gelatin Antibody Prion	5.00	179041.00
Gelatin Antibody Prion	5.00	182117.00
Gelatin Antibody Prion	5.00	175203.80

Table (B-14): Prion detection data in 1.0mg/ml gelatin and 0.3mg/ml SDS solution. Decoy and antibody concentration was 4nM.

Samples	Prion Concentration (nM)	Intensity Values (counts/sec)
Gelatin Control	-	170554.81
Gelatin Control	-	169412.45
Gelatin Control	-	171275.47
Gelatin Antibody	0.00	192725.42
Gelatin Antibody	0.00	184988.00
Gelatin Antibody	0.00	187253.84
Gelatin Antibody Prion	0.50	191487.61
Gelatin Antibody Prion	0.50	185550.27
Gelatin Antibody Prion	0.50	187071.32
Gelatin Antibody Prion	0.80	192400.23
Gelatin Antibody Prion	0.80	187346.15
Gelatin Antibody Prion	0.80	191572.57
Gelatin Antibody Prion	1.00	191296.68
Gelatin Antibody Prion	1.00	185385.57
Gelatin Antibody Prion	1.00	187090.19
Gelatin Antibody Prion	2.00	179967.48
Gelatin Antibody Prion	2.00	172004.53
Gelatin Antibody Prion	2.00	178172.65
Gelatin Antibody Prion	5.00	172992.68
Gelatin Antibody Prion	5.00	168604.72
Gelatin Antibody Prion	5.00	170360.74

Table (B-15): Prion detection data in 2mg/ml gelatin and 0.3mg/ml SDS. Decoy and antibody concentration was 4nM.

Decoy Concentration (nM)	Intensity Values (counts/sec)
163.00	335665.00
140.00	335665.00
116.67	295708.00
93.30	259776.00
70.00	227128.00
23.30	132823.00
18.67	123135.00
16.33	122195.00
11.67	115774.00
9.33	129862.00
7.00	93973.10
4.67	110543.00
0.00	112551.00

Table (B-16): Decoy fluorescence in full strength baby formula.

Decoy Concentration (nM)	Intensity Values (counts/sec)
163.00	811656.00
140.00	707895.00
116.67	607177.00
93.30	489990.00
70.00	393762.00
23.30	75554.00
18.67	59936.00
16.33	48984.00
9.33	35525.00
7.00	25633.00
4.67	13240.00
0.00	0.00

Table (B-17): Decoy fluorescence in 20 times diluted baby formula.

Decoy Concentration (nM)	Intensity Values (counts/sec)		
	40 times Diluted	5000 times diluted	Buffer
163.00	1100000.00	1040000.00	1030000.00
140.00	958895.00	893005.00	733295.00
116.67	815669.00	781505.00	688727.00
93.30	644666.00	608677.00	594912.00
70.00	475565.00	483398.00	441606.00
23.30	109933.00	134795.00	78946.00
18.67	80190.00	103596.00	66312.00
16.33	79063.00	86941.00	46222.00
11.67	61235.00	62958.00	39093.00
9.33	40295.00	52580.00	27989.00
7.00	24449.00	33507.00	25740.00
4.67	19311.00	22150.00	15748.00
0.00	0.00	0.00	0.00

Table (B-18): Effect of baby formula concentration on decoy fluorescence.

Samples	Intensity Values (counts/sec)
Buffer Control	331453.00
Buffer Control	335665.00
Buffer Control	314334.00
Antibody	382126.00
Antibody	397181.00
Antibody	372450.00
Baby formula control	478656.00
Baby formula control	471008.00
Baby formula control	481995.00
Baby formula Antibody	448260.00
Baby formula Antibody	475611.00
Baby formula Antibody	490232.00
Baby formula Triton control	494444.00
Baby formula Triton control	487886.00
Baby formula Triton control	466228.00
Baby formula Triton antibody	576124.00
Baby formula Triton antibody	542712.00
Baby formula Triton antibody	563214.00

Table (B-19): Effect of Triton-X-100 on the percentage differences between control and antibody samples of baby formula.

Samples	Prion Concentration (nM)	Intensity Values (counts/sec)
Baby formula control	-	534371.25
Baby formula control	-	514307.50
Baby formula control	-	541435.00
Baby formula antibody	0.00	693923.80
Baby formula antibody	0.00	690028.80
Baby formula antibody	0.00	685667.50
Baby formula antibody and Prion	2.00	673986.30
Baby formula antibody and Prion	2.00	667712.50
Baby formula antibody and Prion	2.00	664756.30
Baby formula antibody and Prion	5.00	643861.30
Baby formula antibody and Prion	5.00	640205.00
Baby formula antibody and Prion	5.00	672688.80
Baby formula antibody and Prion	10.00	634708.80
Baby formula antibody and Prion	10.00	618557.50
Baby formula antibody and Prion	10.00	600616.30
Baby formula antibody and Prion	15.00	598210.00
Baby formula antibody and Prion	15.00	596346.30
Baby formula antibody and Prion	15.00	592392.50
Baby formula antibody and Prion	20.00	560433.80
Baby formula antibody and Prion	20.00	570728.80
Baby formula antibody and Prion	20.00	569266.30

Table (B-20): Prion detection data in 1.31mg/ml baby formula with 0.454mg/ml Triton-X100.

Samples	Prion Concentration (nM)	Intensity Values (counts/sec)
Baby formula control	-	962211.00
Baby formula control	-	948375.00
Baby formula control	-	942599.00
Baby formula antibody	0.00	1095400.00
Baby formula antibody	0.00	1115100.00
Baby formula antibody	0.00	1097300.00
Baby formula antibody and Prion	2.00	1085300.00
Baby formula antibody and Prion	2.00	1068800.00
Baby formula antibody and Prion	2.00	1070700.00
Baby formula antibody and Prion	5.00	1050100.00
Baby formula antibody and Prion	5.00	1055300.00
Baby formula antibody and Prion	5.00	1062800.00
Baby formula antibody and Prion	10.00	1013600.00
Baby formula antibody and Prion	10.00	1008600.00
Baby formula antibody and Prion	10.00	1004800.00
Baby formula antibody and Prion	15.00	1014200.00
Baby formula antibody and Prion	15.00	1010300.00
Baby formula antibody and Prion	15.00	1006700.00
Baby formula antibody and Prion	20.00	998597.00
Baby formula antibody and Prion	20.00	972208.00
Baby formula antibody and Prion	20.00	988249.00

Table (B-21): Prion detection data in 5.34mg/ml baby formula with 0.454mg/ml Triton-X100.

Sample	Prion Concentration (nM)	Intensity Values (counts/sec)
Decoy	0.00	140021.00
Decoy	0.00	133640.00
Decoy	0.00	136503.00
Decoy and Prion	0.50	141653.00
Decoy and Prion	0.50	139828.00
Decoy and Prion	0.50	143739.00
Decoy and Prion	0.80	138565.00
Decoy and Prion	0.80	141514.00
Decoy and Prion	0.80	130410.00
Decoy and Prion	1.00	132619.00
Decoy and Prion	1.00	126504.00
Decoy and Prion	1.00	128567.00
Decoy and Prion	2.00	137203.00
Decoy and Prion	2.00	137081.00
Decoy and Prion	2.00	141152.00
Decoy and Prion	5.00	147970.00
Decoy and Prion	5.00	132769.00
Decoy and Prion	5.00	142634.00
Decoy and Prion	10.00	139634.00
Decoy and Prion	10.00	136831.00
Decoy and Prion	10.00	139634.00

Table (B-22): Effect of Prion concentration on decoy fluorescence. Decoy concentration was 4 nM and experiment was done in phosphate buffer.

Intensity Values (counts/sec)		
Decoy and Buffer	**Decoy and SDS**	**Decoy and Triton-X-100**
359893.00	360993.00	339610.00
330187.00	369390.00	331484.00
361084.00	367008.00	349127.00

Table (B-23): Effect of buffer, SDS and Triton-X-100 on the decoy fluorescence. Decoy concentration was 10 nM.

Ingredients of Baby formula
Whey protein concentrate (Cow's milk)
Vegetable's oil
Lactose
Corn maltodextrin
Minerals (Potassium citrate, Calcium chloride, Calcium phosphate, Potassium phosphate, Sodium citrate, Magnesium chloride, Ferrous sulfate, Zinc sulfate, Sodium chloride, Copper sulfate, Potassium iodide, Manganese sulfate)
Soy lecithin
Vitamins (Sodium ascorbate, Inositol, Choline bitartrate, Alpha-tocopheryl acetate, Niacinamide, Calcium pantothenate, Riboflavin, Vitamin A acetate, Pyridoxine hydrochloride, Thiamine mononitrate, Folic acid, Phylloquinone, Biotin, Vitamin D_3, Vitamin B_{12}
Taurine
Nucleotides (Cytidine 5-monophosphate, Disodium uridine 5- monophosphate, Adenosine 5-monophosphate, Disodium guanosine 5-monophosphate)
L-cartinine

Table (B-24): Ingredients of Good Start brand Nestle baby formula

VITA

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